Saskatchewan Agriculture, Food and Rural Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20000045

ECOLOGICAL RISK OF TRANSGENIC INSECT RESISTANCE UNDER CANADIAN FIELD CONDITIONS

Funded by:
The Agriculture Development Fund

May 2003
Prepared by: Agriculture and Agri-Food Canada

104-00706/
RECEIVED
MAR 31 2003
REGINA, SASK
S4S 0B1
AGRICULTURE
PROVINCE OF SASKATCHEWAN

Final Progress Report to
Agriculture Development Fund
Project 20000045
31 March 2003

1. Project Title: **Ecological Risk Of Transgenic Insect Resistance Under Canadian Conditions**

2. Applicant (Principal Researcher): Dr. Lorraine Braun
Agriculture and Agri-Food Canada
Saskatoon Research Centre
107 Science Place
Saskatoon, Saskatchewan
S7N 0X2

Collaborators: Dr. P. G. Mason, Dr. S. Warwick, AAFC, Central Experimental Farm, Ottawa, Ontario K1A 0C6; Dr. O. Olfert, AAFC, Saskatoon Research Centre, Saskatoon, SK S7N 0X2

3. Abstract/Summary:

The overall objective of this project is to investigate the movement of insecticidal and "in vivo marker" transgenes from canola (*Brassica napus*) into two closely related weeds and to examine the ecological risk effects of insect resistance to such transgenes under Canadian field conditions. The project was conducted in conjunction with another project of the same name funded by the Canadian Biotechnology Strategy Fund, in which funds were released to complete only part of the experimental work in Saskatchewan. Specific objectives here therefore are to: a) document gene flow between canola and *Brassica rapa* under Saskatchewan conditions; b) develop estimates for the likelihood of insecticidal transgenes to become incorporated into surrounding weed communities and their survival in the presence and absence of selection pressures; and c) determine the likelihood for development of *Bacillus thuringiensis* resistance in diamondback moth populations.

4. Executive Summary

We have completed two years of field and laboratory experiments designed to determine the likelihood of insecticidal transgene movement from *Brassica napus* canola into *Brassica rapa* under field conditions, to examine effects of herbivory on survival of plants containing insecticidal genes, and to monitor the diamondback moth populations for presence of genes for resistance to *Bacillus thuringiensis*. Two lines of GFP (green fluorescent protein) and nine lines of GFP-*Bacillus thuringiensis* (GT) canola (*Brassica napus*) were obtained from Dr. Neal Stewart (University of N. Carolina). These lines were hybridized with three wild accessions of *B. rapa*

populations, F_1 hybrids and BC_1 progeny were screened and individuals showing the presence of the GFP-Bt transgenes were backcrossed onto the appropriate *B. rapa* parent to produce BC_1 and BC_2 respectively. Since the transformed *B. rapa* used in Ontario resulted from a cross of GT canola with naturally occurring weedy populations from Québec and California, BC_2 was not used in the Saskatchewan field trial (it was not permissible to import these lines to Saskatchewan; plant material was made available for laboratory screening bioassays only). GFP-Bt wild radish lines have not been successfully transformed and therefore are not available. In addition, *Raphanus raphanistrum* is limited in canola growing regions of Canada to Québec and Alberta (Warwick *et al.* 2000), and since it does not occur in Saskatchewan, it would not be permitted to import it for field experiments.

All seed stocks were increased and/or additional crosses made prior to further studies in Dr. Warwick's laboratory (Ottawa). Laboratory colonies of diamondback moth from wild populations in western Canada were established.

Field trials were conducted in Saskatchewan (2001, 2002) to determine frequency of outcrossing from GT *B. napus* canola to non-transgenic *B. rapa* bird's rape. Individual plants of *B. rapa* cv. Parkland (in pots) were buried in a 5 m × 5 m plot of GT *B. napus* during flowering. After flowering was complete, the *B. rapa* pots were removed from the field, and placed in a greenhouse for seed ripening. From each individual plant, ca. 200 seeds were sown in the greenhouse and screened for the GFP marker. All putative F_1 hybrids having the GFP trait were evaluated for species-specific morphological traits and up to three hybrids from each *B. rapa* plant were evaluated for pollen viability and ploidy level. A total of 7111 (2881 in 2001; 4230 in 2002) seedlings were screened for the GFP trait in the *B. rapa* AC Parkland × GFP *B. napus* field experiment. The average hybridization frequency detected in 2001 was 7.1%, with a range of 0 to 32.7%. In 2002, the average hybridization rate was 1.2%, with a range of 0 to 5.6%.

Field trials were conducted in Saskatchewan in 2001 and 2002 to determine effects on plant fitness in *B. napus* from the presence of *GFP* and/or *bt* genes. A main plot-sub-plot design was used in which GT canola was grown with or without the presence of the herbivore, diamondback moth. Damage ratings were taken twice during the season (2001) and/or when plants were harvested (2001, 2002). Plants were harvested such that vegetative and reproductive components were kept

separate. All plant material was dried and weighed; data are presented for vegetative and reproductive components, and for the reproductive component expressed as a proportion of total plant weight. Under high insect pressure from the susceptible insect DBM, GT plants had significantly higher mean weight of reproductive tissues than did non-transformed Westar controls.

A total of 868 DBM (eggs, 2nd and 4th instar larvae) were screened for presence of genes conferring resistance to *Bacillus thuringiensis*. At the LD_{95} dose of the commercial formulation of Safer's BTK™ Biological Insecticide (*Bacillus thuringiensis* subsp. *kurstaki*), DBM eggs, 2nd instar larvae and 4th instar larvae had average mortality of 85%, 100%, and 85% respectively (% mortality assessed at 7 days post-treatment). There was no evidence of presence of genes conferring resistance to the *bt* gene.

5. Technical Report

Background:

Genetically modified plants are rapidly becoming commercialized and stabilized in the environment. A primary issue in environmental risk evaluation is the possible escape of transgenes into the natural environment, and the ramifications of gene escape, including the possibility of increased invasiveness and competitiveness of transgenic genotypes compared to weedy wild relatives and the surrounding vegetation (Ellstrand and Hoffman1990; Hoffman 1990; Raybould and Gray 1993; Rissler and Mellon 1996; Snow and Palma 1997). While neutral transgenes (*i.e.* do not confer a fitness advantage or disadvantage) may have minimal ecological effects to their hosts and ecological systems (Crawley *et al.* 1993), a transgene conferring increased fitness may have significant effects (Linder and Schmitt 1994; Schmitt and Linder 1994; Linder and Schmitt 1995; Rissler and Mellon 1996; Snow and Palma 1997). Fitness-conferring transgenes may have their greatest effects when they are in a host that may persist outside of cultivation, and/or may be spread from an agricultural host to a weedy relative. It is conceivable that a crop plant may become weedy or related weeds could become weedier.

The first insect-resistant transgenic crops, including commercially released Bt corn, Bt cotton and Bt potatoes (Kantz 1997), are based on insecticidal proteins from a soil bacterium, *Bacillus thuringiensis* (Bt) (Krattiger 1997). Insect- resistant plants currently under development include both Bt and non-Bt proteins with novel modes of action and different spectra of activity against

insect pests. Development of transgenic canola incorporating Bt δ-endotoxin genes is already underway (Stewart *et al.* 1996; Stewart *et al.* 1997). Stewart *et al.* (1997) have shown that transgenic canola will likely not become more weedy (*i.e.* Bt canola will not invade undisturbed fields and thereby disrupt natural plant communities and ecosystems). However, when defoliating insects are present in moderate to high levels, Bt canola has higher fitness than non-transgenic canola, thus enabling it to persist to a greater degree at field margins and disturbed habitats. Of potentially greater importance, when canola is engineered with fitness enhancing gene(s) and planted on large areas, there will be increased opportunity for outcrossing to weedy wild relatives, and therefore to create potentially weedier weeds.

Diamondback moth has been a periodic but devastating pest of canola in Western Canada during the last 20 years. More than 1.25 M ha were sprayed for diamondback moth in 1995 (WCCP 1994, 1995) resulting in an estimated cost of $200 million to prairie canola growers in 1995. DBM is thought to establish itself in Saskatchewan each year via immigration from the southern United States (Burgess *et al.* 1977). The Saskatchewan population is essentially a dead end population because it does not overwinter here (Harcourt 1960; Butts 1979; Dosdall 2002). Savdie *et al.* (1994) showed that, between the years 1988 to 1992, diamondback moth counts at Saskatoon could be explained in most years by back trajectories of high level winds. In 1997, Agriculture Canada, Environment Canada and the Canola Council of Canada began a further investigation to develop models that would provide an early-warning system for diamondback moth migrations from source areas in the USA and Mexico. Based on wind patterns in 1995 and a knowledge of the source areas in USA and Mexico, Hopkinson (1997) has shown that three-dimensional trajectory models, run in back and forward trajectory mode, can identify regions of the prairies that are most at risk from diamondback moth.

DBM is susceptible to commercial formulations of Bt (Dipel, Xentari), however DBM populations resistant to Dipel are known in the Philippines, Hawaii, China, Japan, Malaysia, and Thailand (Tabashnik *et al.* 1990; Ferré *et al.* 1991; Tanaka and Kimura 1991; Syed 1992; Zhao *et al.* 1993; Whalon and McGaughey 1993), as well as New York and Florida (Shelton *et al.* 1993). Field-collected populations of DBM will be screened to ensure their susceptibility to Bt, and if susceptible, will be used in insect bioassays to detect presence of the transgene marker (Bt-GFP). The trajectory models will be used to help determine source locations for the Saskatchewan

populations, especially if resistance to Bt is detected.

The Bt-GFP canola system is an ideal technology to track spread, persistence and hybridization of a transgenic trait to weedy relatives of canola. Results will be directly applicable to other transgenic strategies developed for canola, including non Bt-based insecticides and herbicide resistance, and could prove valuable in risk assessment studies of transgenic crops. Access to a unique tracking system for transgenic plants, Bt-GFP, may have wider application for measuring gene flow in other *Brassica* crops, as well as in other transgenic crops. Improved data on fitness and gene flow statistics will provide an improved footing for the regulatory system and will benefit the research community by underscoring the environmental significance of genetic modification for insect resistance. The information generated from such funds is necessary to meet current trade/regulatory issues as regards to transgenic plants.

This proposal addresses the deficiency of relevant field data on potential environmental risks of transfer of transgenic traits from crops into weedy relatives. This information is particularly important for risk assessment and risk management decisions in the area of insecticidal transgenes, as this biotechnology has been rapidly adopted by producers in major crop systems. Consumers are increasingly demanding a reduction of pesticide residues in food crops. An improved pest management system for diamondback moth will minimize pesticide use, resulting in reduced producer input costs and will contribute to sustained production of a high quality canola crop. Consumers are also being bombarded with information on the risks of plant biotechnology. Information gathered in this study will enable scientists, regulators and consumers to make informed choices about the potential of escape of transgenes into the natural environment. ADF funding will add to existing expertise by providing technical support for Saskatchewan-based field and laboratory experiments in collaboration with the leading US research group for risk assessment of insecticidal transgenes.

Project Objectives:

Our research plan is to: a) collect and establish cultures of diamondback moths from western Canada; b) assess baseline susceptibility of field populations of DBM to Dipel using laboratory bioassays; c) perform laboratory bioassays to assess mortality of DBM on leaf discs of Bt transgene lines of canola, wild radish and bird rape; d) field test experimental varieties for response

to insect herbivory: non-transformed canola *Brassica napus* L., herbicide tolerant *B. napus*, GFP-transformed *B. napus*, GFP-Bt-transformed *B. napus*; e) field test to monitor possible spread and persistence of transgenes via hybridization to one closely related weedy relative; and f) monitor natural population cycles of DBM and conduct surveys to ascertain early season host plants of DBM. All transgenic canola and canola/weed hybrid lines will be made available to the research team by Dr. Neal Stewart, University of North Carolina (currently located at Dept. of Plant Sciences and Landscape Systems, 2431 Centre Drive, Ellington Plant Sciences, University of Tennessee, Knoxville, Tennessee).

Dr. Stewart will provide three Bt-GFP transgenic lines [canola (*Brassica napus*), bird's rape (*Brassica rapa*) and wild radish (*Raphanus raphanistrum*)] containing a novel whole plant "in vivo marker system" [green fluorescent protein - GFP] and Bt *cry1Ac* which controls the diamondback moth (*Plutella xylostella*). These lines are being developed for large-scale field transgenic risk assessment studies and will allow for the first time assessment under western Canadian field conditions for: i) the fitness of an insecticidal transgene conferring Bt resistance in both the crop and weeds, and ii) the likelihood of its possible spread and persistence via hybridization in two closely related weedy relatives [both bird rape and wild radish are weeds of agricultural fields and are able to cross with canola under field conditions]. Only *B. rapa* was used as a 'weed' in Saskatchewan. *Raphanus raphanistrum* research was carried out in Ottawa.

Project Activities and Achievements (to March 31 2003):

1. **Collect and establish cultures of diamondback moths from western Canada; monitor natural population cycles of DBM and conduct surveys to ascertain early season host plants of DBM**

Diamondback moths were collected from various populations in Saskatchewan in 2001 and from the Saskatoon AAFC farm in 2002, and laboratory cultures were established (see Table 1 for collection locations).

Diamondback moth was a serious pest in the canola growing region of Saskatchewan in 2001, appearing in early May and producing from 3 to 4 generations through the summer, which resulted in staggered populations. The DBM were highly parasitized in some areas, resulting in up to 65%

reduction in larval population due to parasitism. Nevertheless, approximately 2.1 M ha were sprayed to control DBM in SK in 2001 (S. Hartley pers. comm.). Collections were made near the Maple Creek/Leader area, at Naicam, and near the Scott/Unity area. Colonies established in the laboratory from each location were maintained separately in order to assess each for rates of parasitism and presence of Bt resistance genes. In western Canada, three species of parasitic wasps attack the diamondback moth. *Diadegma insulare* (Cresson) and *Microplitis plutellae* (Muesebeck) attack the larval stages while the third species, *Diadromus subtilicornis* (Gravenhorst), attacks the prepupal and pupal stages.

Drought conditions throughout the canola growing regions of Saskatchewan in 2002 delayed plant germination and establishment (of both crop and native plants). DBM was not a pest of canola in 2002 in any region of Saskatchewan. High level wind trajectories from the southern USA and Mexico were examined as possible indicators of DBM migration from source populations. In early June 2002, wind trajectories showed movement of high level winds from Mexico to the Spiritwood and Chitek Lake areas, however no DBM were found in subsequent sampling of canola and weeds in these areas. DBM larvae were collected from several locations in western Saskatchewan, however numbers were too low to establish laboratory colonies. Forty-two rural municipalities were sampled in July 2002 but only 574 larvae and 20 adult DBM were collected from all samples (Table 2). Host plants included canola, mustard, volunteer mustard, flixweed (*Descurainia sophia* (L.)), and bird's rape (*B. rapa* L.). One laboratory colony was established from DBM collected in 2002 in a canola nursery at the Saskatoon Research Centre farm.

The diamondback moth feeds only on members of the family Cruciferae. DBM can maintain itself on some cruciferous weeds only in the absence of more favoured cultivated hosts, such as canola, mustard, cabbage and other cruciferous vegetables. The following crucifers which have been reported to sustain feeding and reproduction of diamondback moth occur in Saskatchewan: bird rape, *Brassica rapa* L., small-seeded false flax, *Camelina microcarpa* Andrz., Shepherd's purse, *Capsella bursa-pastoris* (L.) Medic., heart-podded hoary cress, *Cardaria draba* (L.) Desv., hare's-ear mustard, *Conringa orientalis* (L.) Dumort, flixweed, *Descurainia sophia* (L.) Prantl, ball mustard, *Neslia paniculata* (L.) Desv., wild mustard, *Sinapsis arvensis* L., tumble mustard, *Sisymbrium altissimum* L., and stinkweed, *Thlaspi arvense* L. Alternate weed hosts are especially important in maintaining diamondback moth populations in temperate countries in spring before

cruciferous crops are planted. In 2001, DBM populations arrived early in the season and were able to infest early-seeded canola directly. In 2002, DBM were collected in forty-two RMs (development of both crop and weedy plants was delayed due to drought conditions over most of the province).

The wild cruciferous species likely to be present close to the research plots in Saskatoon include wild mustard, *Sinapis arvensis* L., stinkweed, *Thlaspi arvense* L., and shepherds purse, *Capsella bursa-pastoris* (L.) Medic. Although stinkweed and shepherd's purse are distantly related to oilseed rape, the probability of crossing is virtually non-existent.

2. Assess baseline susceptibility of field populations of DBM to Dipel[1] using laboratory bioassays

Baseline susceptibility of field populations of DBM to a commercial formulation of Bt has been determined through laboratory feeding bioassays (Table 1; Fig. 3). The formulation used was Safer's BTK™ Biological Insecticide (*Bacillus thuringiensis* subsp. *kurstaki*) [Safer Ltd., Box 186, Scarborough Ontario) (*Bacillus thuringiensis* sub-species *kurstaki*; Potency 10,600 IU/mg equivalent to 12.6 Billion International Units per litre)]. The colonies established from Maple Creek, Naicam and Wilkie/Scott in 2001, and Saskatoon in 2002 reacted as expected to Bt in feeding bioassays, with no evidence of resistance to BTK, a commercial formulation of Bt commonly used in the US and Mexico to control DBM. Based on population response to the LD_{95} dose at Day 6 post-treatment, there was no evidence of Bt-resistance genes in DBM populations collected in Saskatchewan.

3. Perform laboratory bioassays to assess mortality of DBM on leaf discs of Bt transgene lines of canola, wild radish and bird rape

Wild radish (*Raphanus raphanistrum*) has not been successfully transformed with GFP-Bt and therefore lines were not available for experimentation.

---

[1] We used Safer's BTK™ instead of Dipel. Both are derived from *Bacillus thuringiensis* subsp. *kurstaki*, and contain several *bt* genes, including *cry1Ac*.

Canola (*Brassica napus* cv Westar) was transformed with two GFP (green fluorescent protein) constructs, *mGFP5er* (GFP only) and *pSAM 12* (GFP linked to a synthetic *Bacillus thuringiensis cry1Ac* gene; lines GT 1-10) (Halfhill *et al.* 2001a) and was supplied by Dr. Neal Stewart, University of North Carolina. Nine lines of GFP-Bt (GT) and two lines of GFP canola were selfed and progeny screened in the laboratory of Dr. Warwick to obtain homozygous lines. *Brassica napus* cv. Westar and cv Excel were used as controls.

The nine GT and two GFP *B. napus* lines were hybridized with three wild accessions of *B. rapa* (one California (CA) and two Québec (QB1 and QB2) populations (Halfhill *et al.* 2001). F_1 hybrids and BC_1 progeny were screened and individuals showing the presence of the GFP-Bt transgenes were backcrossed onto the appropriate *B. rapa* parent (CA or QB1 and QB2) to produce BC_1 and BC_2 respectively. Seed increases and hybridizations were performed in the lab of Dr. Warwick, Ottawa. BC_2 was used in the Ontario field trial only (since the transformed *B. rapa* used in Ontario resulted from a cross of GT canola with naturally occurring weedy populations from Québec and California, it was not permissible to import these lines to Saskatchewan). QB1 backcross line was not tested in Saskatchewan.

A total of 26 feeding bioassays using 16 different transgenic GFP-Bt (=GT) *B. napus* lines and 2 lines of *B. napus* containing GFP alone were conducted on fourth instar (n=12) and eggs (n=14) of the western population diamondback larvae between October 2000 and December 2002 in Saskatoon (Tables 3, 4). Twelve bioassays using transgenic *B. rapa* were conducted against fourth instar (n=6) and eggs (n=6) of diamondback (Tables 5, 6). Transgenic *B. rapa* lines tested included 3 lines originating from backcrosses with the California population (CA BC_1, CA BC_2a, CA BC_2b) and 2 lines originating from backcrosses with the Québec QB2 population (QB2 BC_2a, QB2 BC_2b). Bioassays on *B. rapa* lines were conducted on fresh leaf material sent from Ottawa (this plant material was not grown in SK) and amount of plant material was limited, so only a minimum of assays were performed, and these concluded when all leaf material was consumed.

Preliminary assays were performed to determine the LD_{50} and LD_{95} values[2] of Safer's BTK™

[2] LD_{50} and LD_{95} are the Lethal Doses of a substance required to kill 50 and 95% of the population tested, respectively.

Biological Insecticide (*Bacillus thuringiensis* subsp. *kurstaki*) against western populations of DBM. Controls were comprised of canola leaf disks (12.5 mm diam) either untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK, allowed to air dry for 15 minutes, and placed on 1.2% agar. Treatments included leaf disks from GFP, nine GT *B. napus* lines, *B. rapa* parental lines from California and Québec, BC_1 and BC_2 lines from California, and BC_2 lines from Québec. Controls were appropriate for the species tested: cv Westar used for *B. napus* assays; CA or QB2 lines used for *B. rapa* assays. Leaves were placed on 1.2% agar in 12 well plates. One diamondback egg or larvae was added to each well (1 insect per leaf disc, n=24 or n=12 respectively). Plates were sealed with Mylar, aerated, and maintained at 22°C ± 2°C, 50% ± 2% RH, and a 16:8 light:dark photoperiod. In egg bioassays, only those eggs which hatched were included in the analysis. Leaf damage and mortality were scored daily for up to 14 or 21 days after the test was set up (larvae and eggs respectively). Leaf damage was assessed using a 0 - 10 scale (5 = 50% of the leaf consumed). Cumulative damage ratings were used to calculate total leaf consumption over the test period. Analysis of variance was performed using the general linear model procedure, and differences between means were tested for significance with Waller-Duncan t-test (P=0.05) (SAS Institute 1985). Comparisons between each treatment were made for % mortality and total leaf consumption (mm^2) and of individual larvae.

DBM mortality (for both eggs and larvae) was significantly higher for insects fed *B. napus* GT lines containing the *bt* gene than for DBM fed untreated Westar controls at any time period post-treatment (Tables 3 and 4). The lines transformed with GFP alone [GFP-2(1), GFP-2(2)] did not result in higher mortality to DBM 4^{th} instar larvae than untreated canola (Table 4). Mortality in DBM eggs fed with GFP-2(2) was only significantly different from untreated Westar controls at 18-21 days post-treatment (Table 3). Total plant area consumed over 14 or 21 days post-treatment was significantly lower for both larvae and eggs (respectively) fed GT lines than for those fed untreated Westar controls or *B. napus* transformed with GFP alone.

DBM mortality was significantly higher for insects (both eggs and larvae) fed *B. rapa* BC_1 or BC_2 GT lines containing the *bt* gene than for DBM fed untreated parental control lines (CA, QB2) at any time period post-treatment (Tables 5 and 6). Total plant area consumed was significantly lower for both larvae and eggs fed *B. rapa* BC_1 or BC_2 GT lines derived from the CA *B. rapa* population. Leaf material from the Québec *B. rapa* QB_2 backcross lines was limited, and bioassays were

conducted only against the egg stage. Total consumption for eggs fed QB2 'wet' control was not significantly different than for eggs fed with backcross GT lines derived from the Québec *B. rapa* population QB2 at 10 - 14 days post-treatment (Table 6).

For both plant species, mortality of DBM eggs fed with transgenic leaf disks containing the Bt gene was 100% by 10-14 days post-treatment. DBM larvae fed with transgenic *B. napus* had a mean mortality of 78%, and leaf area consumed was only 1.3 mm^2 over a period of 1 week. Fourth instar DBM larvae fed with transgenic *B. rapa* had 78% mortality, with mean consumption of 9.6 mm^2 at one week post-treatment. Although insects treated with Bt do not die immediately, feeding is severely restricted after Bt intoxication.

Data for 10 - 14 days post-treatment showed a significant increase in total leaf area consumed for plant lines used as 'controls': Westar, CA, QB2 and GFP.

This is the first reported use of the egg stage of DBM in a Bt laboratory feeding bioassay. The egg stage is the most applicable stage to test in field situations, since DBM moths are attracted to and will oviposit directly on a suitable cruciferous host plant (weed or crop). Eggs hatch and 1st instar larvae immediately enter the leaf to live as leaf minors within the leaf tissue. Ingestion of the first bite of any GT plant line in the tunnelling process was apparently sufficient to kill all DBM larvae which started a bioassay as eggs. (In most insects, Bt intoxication is higher in younger instars.) Chances of a 4th instar larva moving in the plant canopy from a non-transgenic plant to one containing the *bt* gene are reduced as DBM larvae do not move great distances within a crop or plant canopy. Therefore DBM would be killed as 1st instar larvae if eggs were laid on transgenic plants containing the *bt* gene.

4. Field test experimental varieties for response to insect herbivory: non-transformed canola *Brassica napus* L., herbicide tolerant *B. napus*, GFP-transformed *B. napus*, GFP-Bt-transformed *B. napus* (with excellent, good and poor fluorescence)

NOTE: the designations of excellent, good and poor fluorescence were used in preliminary experiments in 2000 as a method of quantifying plants within lines of GT canola. It was later discovered that this characterization was not significant, as variations in the expression of

fluorescence due to GFP was due to plant growing conditions and visual screening methods, and did not reflect differences in expression of the *bt* gene. The excellent, good and poor designation was not used in 2001 and 2002.

NOTE: Herbicide tolerant (HT) canola was not included in the Saskatoon experiments due to size limitations of the field plot and the number of available insect cages.

Drought conditions in the canola growing regions of Saskatchewan in 2002 impacted on all aspects of the field experiments. Irrigation was required prior to seeding to ensure seedling germination and establishment in both 2001 and 2002. However the screened cages provided some shading and provided excellent growth conditions for plants during periods of high temperature.

Herbivory experiment (Bt-GFP *B. napus* lines planted within cages, with or without diamondback moth added). All experiments conducted according to CFIA regulations for Confined Field Trials for Plants With Novel Traits.

In 2001 and 2002, thirty-two 3 m^2 plots were established, each containing two 1 m^2 seeded areas; a randomized main plot-sub-plot design (with DBM added; without DBM added) with 4 replicates was used (Figures 1, 2, 3, 4). Seeded areas contained canola as follows: 4 plots of non-transgenic *B. napus* cv Westar, 4 plots of GFP *B. napus* canola, and 24 plots of GT *B. napus* canola (in 2001: GT-1(2), GT-4(7), GT-5(1), GT-6(3), GT-7(2), GT-8(10); in 2001 only GT-6(3) was used). The 64 1 m^2 plots were seeded by hand at 40 seeds/m^2 [seeded 22 May 2001, 21 May 2002]. The area outside each cage within the plot was unseeded. Cages were installed in each plot after seeding between seedling to rosette stage (2001) (stage 1 - 2, Harper and Berkenkamp 1975) or before seedling emergence (2002). All seed was treated with Vitavax RS Flowable at the rate of 0.562 L/25 kg seed for flea beetle control. Plots were treated with pre-emergent Edge granular (ethafluralin) (Dow AgroSciences) at 20 lbs/ac for weed control. The land was treated the pervious fall with N, P, K, S fertilizer 65-35-10-15 at 228.74 lbs./ acre (2001) and 45-40-10-15 at 195.32 lbs./ acre in 2002.

DBM larvae were added as available to each DBM-treatment cage (150 larvae per cage from 11 July to 2 August 2001; 72 larvae and 12 adults per cage from 26 June to 14 August 2002). Damage

ratings in control cages and in 4 GT canola cages were taken twice during the season (2001) and/or when plants were harvested (2001, 2002). Plants in cages were harvested by cutting stems off at ground level while pods were green. Plant counts were taken in each cage; ten plants that had been previously rated for damage (2001) or ten random plants (2002) were bagged individually into paper bags, reproductive components in one bag and vegetative components in another bag. Reproductive components included all flowers, pods, and stems bearing flowers or pods. Remaining plants from each cage (to a total of 30 plants) were bulked into reproductive and vegetative components. If more than 30 plants were left in the cage after the 10 tagged plants were removed, smaller and weaker plants were discarded, as they were likely the result of late germination. All plant material in paper bags was dried and weights recorded. All plant material was destroyed by burial at the end of the test as per CFIA regulations.

Weight of vegetative and reproductive plant components, and reproductive components expressed as proportion of total plant weight for individual plants, and weights for all plants per cage in each treatment are shown in Tables 7 (2002) and 8 (2001). In 2002, DBM pressure was considered 'high' whereas is 2001, pressure was 'low'. High herbivore pressure in 2002 field test resulted in a significant increase in both the reproductive weight and the proportion of the total weight comprised by the reproductive component (Table 7) of GT plants for both individual plants and total cage weight. The *bt* gene conferred increased fitness (as measured by the plant's ability to reproduce) to plants under high insect pressure from the susceptible insect, DBM.

Mean individual plant weight (reproductive component) of untreated Westar or GFP plants grown under high insect pressure was significantly lower than for plants grown under zero pressure, whereas plant weight of GT-6(3) plants grown under zero or high DBM pressure did not differ (Student's paired t-test comparing mean plant weights for plants grown under zero and high pressure (in rows).

Low insect pressure in 2001 did not provide sufficient herbivory to differentiate between untreated control plants and GT lines, or between zero and low insect pressure (Table 8).

It appears that GT *B. napus* plants in 2002 were more fit (*i.e.* have higher total weight per plant, higher reproductive weight per plant) than untreated Westar. Line GT-6(3) provided excellent

resistance to DBM: presence of DBM had no effect on mean plant weight (reproductive component) in 2002. The addition of DBM to cages earlier in the growing season in 2002 was likely the reason for the improved performance in the field test. The insects were able to establish a population early in cages and have an effect on plant growth and reproductive potential.

5. Field test to monitor possible spread and persistence of transgenes via hybridization to one closely related weedy relative

Outcrossing experiment (Bt-GFP *B. napus* pollen flow to non-transgenic *B. rapa*). Significant changes in field experiment methodology were made to accommodate AAFC and CFIA policy on growing weedy lines of *B. rapa* on AAFC land.

One field plot was set up for the gene flow experiment at the AAFC Research farm at Saskatoon SK (see Figures 1 & 2, Plot Plans 2001, 2002): a 5 m × 5 m plot of GFP-Bt canola was seeded (23 May 2001; 21 May 2002) using a Hege small plot seeder which has 100% delivery of seed (no cleanout is required). This plot was separated from the remaining field plots by a distance of 4 feet. All seed was treated with Vitavax RS Flowable at the rate of 0.562 L/25 kg seed to control flea beetles. *Brassica rapa* acted as the 'weed' and was planted at a density of 1 plant per m^2. This ratio reflects a realistically low population of weed in relation to crop, and will provide a situation in which more canola pollen will be available for weed pollination relative to weed pollen.

B. rapa cv. AC Parkland was planted in pots over several weeks and grown in the greenhouse at the Saskatoon Research Centre. When the GFP-Bt *B. napus* in the 5 ×5 m plot was flowering, 25 flowering *B. rapa* plants, still in their pots, were transplanted into the plot at regular spacing (see Fig. 6). After flowering was complete (on 7 August 2001, 8 August 2002), the *B. rapa* plants (in pots) were removed from the field, and placed in a greenhouse at AAFC-Saskatoon Research Centre. All stems bearing pods were bagged to contain any seeds before removal from the field. The transgenic GFP-Bt *B. napus* plants in the 5 × 5 m plot were then harvested by hand (by cutting plants off at ground level), bagged and placed in storage to await disposal. The potted *B. rapa* plants were allowed to set seed, and seed was harvested on a per plant basis.

Seeds were screened to determine the frequency of outcrossing from *Brassica rapa* × *Brassica*

napus. Seeds from each weed plant were germinated in growth chamber or greenhouse. Because we used the GFP trait as an *in vivo* tag, interspecific hybrids were detected by green fluorescence under UV light. This enabled screening of many more hybrids compared with the screening of seed on antibiotic-containing media or any other established method. Plants testing positive for GFP were then examined for presence of hairs (morphological trait of *B. rapa*) as another determination of hybrid status. The average hybridization frequency detected in 2001 was 7.1%, with a range of 0 to 32.7%. In 2002, the average hybridization rate was 1.2%, with a range of 0 to 5.6% (Table 9). All putative F_1 hybrids were grown for use in laboratory feeding bioassays against DBM to test for presence of Bt (in all cases to date the Bt and GFP genes co-occur). All putative F_1 hybrids killed DBM eggs and larvae in feeding bioassays (Tables 10-13). Hybrids from the outcrossing experiment were not retained once bioassays were completed. Mortality was significantly higher for F_1 hybrids than for non-transformed Parkland for both eggs and 4th instar larvae for hybrids obtained in both years. Total leaf area consumed was significantly higher for non-transgenic Parkland than for putative F_1 hybrids for both eggs and 4th instar larvae in 2002 and 2001.

Pollen viability was assessed as the percentage of pollen stained by a 1% aceto-carmine solution. In 2001, a total of 39 *B. rapa* × *B. napus* F_1 hybrid plants were analyzed, with ca. 500 pollen grains examined per plant, for a total of 44104 pollen grains analysed; in 2002, 26 *B. rapa* × *B. napus* hybrid plants were analyzed, with 36407 pollen grains examined (Table 14, Fig. 7). Mean pollen viability ranged from 0.8 to 97.2% in 2001 and 3.2 - 58.8% in 2002, for an overall mean of 42.6%.

Ploidy levels were assessed from flow cytometric data using a Partec PA-I Flow cytometer which measures relative amounts of DNA (Dolezel 1991). About 0.5-1 cm^2 of leaf material was chopped with 0.5 ml of lysis buffer, filtered through a 50 µm nylon mesh filter, and combined with ca. 2 ml of the staining solution containing DAPI. The analysis was performed using a Partec PA-I equipped for UV excitation and blue light emission. At least 8000 nuclei were analyzed in each sample and the histogram mean value for G1 peaks evaluated. Intermediate cytometric values were expected for putative diploid and triploid hybrids; all samples had values intermediate between the two parents, *B. napus* and *B. rapa* (data not shown).

6. Additional activities

In 2001 we contacted Joanne Buth and Managers of the Canola Production Centres, Phil Thomas

of Alberta Agriculture, Bill Greuel of SAF and Drs. Gord Thomas and Hugh Beckie of AAFC Saskatoon, with a request for help in locating areas where HT canola (*B. napus*) occurs in close proximity with volunteer or escaped *Brassica rapa* populations. Each contact passed on our request to managers, field and crop specialists and extension agrologists, with the hope that sites identified would be visited by us for collection of seed. No such sites were identified in 2001.

Volunteer *B. napus* occurred as a weed in 0.8% of the 631 fields sampled in Manitoba in 2002, in 0.7% of the 153 canola fields sampled, in 1.8% of the 112 oat fields and in 2.7% of the 37 flax fields sampled (Leeson *et al.*, 2002). It had highest occurrence in the Aspen Parkland Ecoregion (6.3% frequency in all flax fields sampled n=16). An extensive weed survey is planned for Saskatchewan in 2003 (Leeson *et al.*, 2002). The region in Saskatchewan likely to have hybridization sites would be limited to areas where *B. rapa* is primarily grown and there are *B. rapa* volunteers - for example in the Medstead area. In 2002, colleagues working under another project funded by the Canadian Biotechnology Strategy Fund identified a barley forage field at Medstead SK (Rm 497) as a site for potential hybridization of HT canola and *Brassica rapa*. They sampled the field and seeds are being analyzed. An early frost at the beginning of August will have a high impact on seed quality. Due to the large time commitment required for screening putative hybrid seeds, we did not attempt to sample and process seeds (as the grant was scheduled to terminate in Dec. 2002).

6. Personnel

Stephanie Ethier occupied the EG02 technical position, initially co-funded by the CBS grant. Cherie Perepelitza and Anita Stadnyk assisted with field research in 2001 and 2002 respectively.

7. Equipment

No equipment was purchased or rented with project funds.

8. Project Developed Materials

One poster on the joint CBS-ADF project was presented at the Society for Invertebrate Pathology annual meeting. August 2001, Noordwijkerhout, The Netherlands. [copy attached]

Braun, L.,Warwick, S., Mason, P., Zhu, B., and Stewart Jr., N. 2001. Ecological risk of transgenic insect resistance under Canadian field conditions - Field and Laboratory Assessment of Fitness and

Toxicity of Bt Canola. Poster. Abstracts p. ABS9.

9. Project Photos

Photographs taken in the field plots at the Saskatoon Research Centre farm in 2001 were attached to the January 2001 report. Additional photographs included here are:

Fig. 3- DBM feeding bioassay showing response to a control (Westar 'dry') and Bt (Westar LD_{95} dose).

Fig. 4- Saskatoon Field Plot 2001 showing the 10 m pollen trap strip, the paired DBM cages in each plot (with/without DBM), and the 5 ×5 m Outcrossing plot.

Fig. 5- 2002 Saskatoon Herbivory Experiment, with Field Plot showing a GFP plot with and without DBM pressure, and a GT-6(3) plot with and without DBM pressure.

Fig. 6- Saskatoon 2001 Outcrossing Experiment; whole plot and close-up view.

Fig. 7- Pollen Viability from Putative *Brassica rapa* × *Brassica napus* F_1 hybrids.

10. Acknowledgement

Producers and other researchers were told verbally that this work is supported by ADF from Saskatchewan. The ADF was acknowledged as a source of funding on the poster.

References

Burgess, L., J. Dueck, G.A. Petrie and L.G. Putnam. 1977. Insect Pests and diseases of rape and mustard. Rapeseed Association Publication No. 48, 32 p.

Butts, R.A. 1979. Some aspects of the biology and control of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Southern Ontario. M.Sc. Thesis, University of Guelph, Guelph, Ontario, 97 p.

Crawley, M.J., R.S. Hails, M. Rees, D. Kohn and J. Buxton, 1993. Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620-623.

Dolezel J. 1991. Flow cytometric analysis of nuclear content in higher plants. Phytochem Anal 2:143-154

Dosdall, L. M., P. G. Mason, O. Olfert, L. Kaminski, and B. A. Keddie. 2002. The origins of infestations of diamondback moth, *Plutella xylostella* (L.), in canola in western Canada. Proceedings of the Fourth International Workshop on the Management of Diamondback Moth and Other Crucifer Pests, Melbourne, Australia. In Press.

Ellstrand, N.C. and C.A. Hoffman. 990. Hybridization as an avenue of escape for engineering genes,. Bioscience 40: 438-442.

Hoffman, C.A. 1990. Ecological risks of genetic engineering of crop plants. Bioscience 40: 434-437.

Harcourt, D.G. 1960. Biology of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in eastern Ontario III. Natural enemies. The Canadian Entomologist 92:419-428.

Hopkinson, R.F. 1997. Weather patterns and the occurrence of diamondback moth on the Canadian Prairies. Interim Report to Canola Council of Canada. 57 pp.

Kantz, B. 1997. Bt crops: Year two. AgConsultant 53:21-22.

Krattiger, A.F. 1997. Insect resistance in crops: A case study of *Bacillus thuringiensis* and its transfer to developing countries. ISAAA (International Service for the Acquisition of Agri-Biotech Applications) Briefs 2:1-42

Linder, C.R. and J. Schmitt. 1995. Potential persistence of escaped transgenes: performance of transgenic, oil-modified *Brassica* seeds and seedlings. Ecol. Appl. 5: 1056-1068.

Linder, C.R. and J. Schmitt. 1994. Assessing the risks of transgene escape through time and crop-wild hybrid persistence. Molecular Ecology 3: 23-30.

Raybould, A.F. and A.J. Gray, 1993. Genetically modified crops and hybridization with wild relatives: a UK perspective. J. Appl. Ecol. 30: 199-219.

Rissler, J. and M. Mellon, 1996. The ecological risks of engineered crops. The MIT Press, Cambridge, Massachusetts.

Savdie, I., J. Stewart and J. Soroka. 1994. Use of Atmospheric Environment Service three-dimensional trajectory model in the development of a near-real time pest alert system. Unpublished manuscript. 21pp.

Schmitt, J. and C.R. Linder. 1994. Will escaped transgenes lead to ecological release? Molecular Ecology 3: 71-74.

Ferré, J., M. D. Real, J. Van Rie, S. Jansens, and M. Peferoen. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proc. Natl. Acad. Sci. 88:5119-5123.

Julia Y. Leeson, A. Gordon Thomas, Todd Andrews, Kim R. Brown and Rene C. Van Acker. 2002. Manitoba Weed Survey. Cereal and Oilseed Crops 2002. Weed Survey Series Publication 02-2. Published by Agriculture and Agri-Food Canada, Saskatoon Research Centre, December 2002.

Shelton, A. M., J. L. Robertson, J. D. Tang, C. Perez, S. D. Eigenbrode, H. K. Preisler, W. T. Wilsey, and R. J. Cooley. 1993. Resistance of diamondback moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. J. Econ. Entomol. 86:697-705.

Snow, A.A. and P.M. Palma, 1997. Commercialization of transgenic plants: potential ecological risks. Bioscience 47: 86-96.

Stewart, C. N., Jr., J. N. All, P. L. Raymer, and S. Ramachandran. 1997. Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. Molec. Ecol. 6:773-779.

Stewart, C. N., Jr., M. J. Adang, J. N. All, P. L. Raymer, S. Ramachandran, and W. A. Parrott. 1996. Insect control and dosage

effects in transgenic canola containing a synthetic *Bacillus thuringiensis crylAc* gene. Plant Physiol. 112:115-120.

Syed, A.R. 1992. Insecticide resistance in diamondback moth in Malaysia. In: Diamondback moth and other cruciferous pests. Proceedings of the Second International Workshop, Asian Vegetable Research and Development Centre, Taiwan. N.S. Talekar, ed.

Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83:1671-1676.

Tanaka, H. and Y. Kimura. 1991. Resistance to *B. thuringiensis* formulation in diamondback moth, *Plutella xylostella* L. on watercress. Jpn. J. Appl. Entomol. Zool. 35:253-255

Warwick SI, A. Francis, J. La Fleche. 2000. Guide to Wild Germplasm of Brassica and Allied Crops (tribe Brassiceae, Brassicaceae) 2nd Edition. Agriculture and Agri-Food Canada Research Branch Publication, ECORC Ottawa, Canada. Contribution No. 991475. [http://res2.agr.ca/ecorc/cwmt/tech.htm#guide]

Western Committee on Crop Pests. 1995. Minutes of the 34th Annual Meeting, October 19-21, 1995. Victoria, British Columbia.

Western Committee on Crop Pests. 1994. Minutes of the 33rd Annual Meeting, October 12-14, 1994. Edmonton, Alberta. 43 pp.

Whalon, M. E., and W. H. McGaughey. 1993. Insect resistance to *Bacillus thuringiensis*. In: Advanced Engineered Pesticides. L. Kim (ed.) Marcel Dekker, Inc., New York.

Zhao, J.-Z., G.-R. Zhu, Z.-L. Zhu, and W.-Z. Wang. 1993. Resistance of diamondback moth to *Bacillus thuringiensis* in China. Resistant Pest Management 5(2), 11-12

Table 1. Rate of parasitism and % mortality of 2nd instar diamondback moth, *Plutella xylostella*, collected in Saskatchewan in 2001 and 2002. DBM second instar larvae were fed *B. napus* cv Excel leaf disks which were dipped in the LD_{50} or LD_{95} rate of Safer's BTK. Data for Day 6 post-exposure. [LD_{50} and LD_{95} are the Lethal Doses of a substance required to kill 50 and 95% of the population tested, respectively.]

		Parasite			Toxicity to BT	
		Microplitis plutellae	*Diadegma insulare*	*Diadromus subtilicornis*	LD_{50}	LD_{95}
Date	**Location**	**Rate of Parasitism**			**% Mortality Day 6**	
01-Jun-01	Maple Creek/Leader	20%	10%		75%	100%
05-Jun-01	Naicam	17%	2%		33%	100%
	Field 1 - N52° 31.611' W 104° 24.295'					
	Field 2 - N52° 32.965' W 104° 24.296'					
	Field 3 - N52° 30.530' W 104° 35.806'					
25-Jul-01	Wilkie/Scott	25%	25%	5%	25%	100%
	Field 1 - NW 20-40-20-W3					
	Field 2 - NW 30-39-20-W3					
30-Jul-01	Wilkie/Scott	30%	30%	5%	25%	100%
	Field 1 - SE 24-39-20-W3					
	Field 2 - NE 31-38-19-W3					
	Field 3 - NW 35-37-20-W3					
36-Jul-02	Saskatoon AAFC Farm	0%	0%	0%	21%	100%

Table 2. Diamondback moth collected in Saskatchewan in July 2002 (42 rural municipalities sampled). (n/a not recorded)

Site	RM	Legal Land Description	Plant Collected From	Growth Stage	DBM Larvae	DBM Adults
2	RM 105	SE 12 11 8 W3	Mustard	4	3	
1	RM 105	SE 13 12 8 W3	Mustard	4	2	
3	RM 105	SE 26 10 9 W3	Napus	4	4	
8	RM 106	NW 17 12 12 W3	Mustard	4	1	
4	RM 106	NW 33 11 9 W3	flixweed		4	
5	RM 106	SE 14 12 10 W3	Volunteer canola	4	11	
6	RM 106	SE 20 10 11 W3	Mustard	4	21	1
23	RM 107	NE 20 10 15 W3	flixweed		3	
26	RM 107	SW 22 12 14 W3	Mustard	4	11	
24	RM 107	NE 31 10 14 W3	Mustard	4	42	
19	RM 108	SE 6 11 18 W3	Napus	4	11	
20	RM 108	SE 9 11 17 W3	Mustard	4	8	
22	RM 108	SE 26 9 16 W3	n/a		11	3
18	RM 109	SW 23 12 20 W3	Napus	4	8	
17	RM 109	SW 34 11 21 W3	Napus	4	51	
16	RM 109	NW 21 10 19 W3	Napus	4	8	2
10	RM 110	NE 16 11 22 W3	Mustard	4	37	
11	RM 110	NW 26 10 23 W3	flixweed & volunteer canola		1	
9	RM 110	SE 28 12 22 W3	flixweed		1	
12	RM 110	SW 2 13 21 W3	flixweed		5	
13	RM 111	NE 33 11 26 W3	flixweed		3	
5	RM 142	NW 27 16 29 W3	Canola	4	4	
6	RM 142	SE 27 16 29 W3	Canola	4	8	
27	RM 165	SE 16 18 8 W3	Canola	n/a	1	
25	RM 165	SE 9 17 9 W3	Volunteer mustard & canola	n/a	1	
24	RM 166	NE 26 17 11 W3	Canola	n/a	10	
14	RM 168	NE22 17 16 W3	Canola	4	5	
13	RM 168	NW 24 17 16 W3	Volunteer canola	n/a	3	
15	RM 168	SW 13 17 17 W3	Canola	5	1	
18	RM 169	NE 12 18 21 W3	Canola	5	26	
17	RM 169	SE 18 17 20 W3	Canola	5	9	
17	Rm 169	SE 18 17 20 W3	Canola	5	9	
9	RM 171	NW 32 17 26 W3	Canola	5	8	
8	RM 171	SW 31 17 26 W3	Mustard in oats	n/a	1	
15	RM 211	NW 14 7 25 W3	flixweed		19	
23	RM 255	NE 34 24 8 W3	Mustard	4	5	
24	RM 255	SW 17 25 7 W3	Canola	4	5	
22	RM 255	SW 33 24 9 W3	Brassica rapa	4	5	
21	RM 256	NE 11 25 11 W3	Volunteer mustard	4	5	
20	RM 256	SE 29 25 11 W3	Volunteer mustard	4	2	
19	RM 256	SE 6 26 12 W3	Mustard	4	1	
15	RM 257	SE 14 26 16 W3	Mustard	4	5	
18	RM 257	SW 20 25 13 W3	Volunteer mustard	4	32	
16	RM 257	n/a	Volunteer mustard	4	2	
14	RM 259	NE 13 25 18 W3	Mustard	4	6	

12	RM 259	NE 29 26 21 W3	Mustard	n/a	14	
11	RM 259	SW 32 26 20 W3	Mustard	4		3
6	RM 260	SW 5 26 24 W3	Volunteer mustard	4	3	
10	RM 260	NW 19 26 24 W3	Canola	5	10	
5	RM 261	SE 25 24 25 W3	Volunteer mustard & canola	4	3	
7	RM 261	SW 18 25 26 W3	Canola	4	2	
2	RM 292	NE 19 30 27 W3	Canola	4	15	
1	RM 292	NE 8 29 27 W3	Canola	4	5	
4	RM 292	SE 17 28 28 W3	Mustard	4	5	
3	RM 292	SW 28 30 27 W3	Mustard	4	25	
8	RM 316	NW 22 32 11 W3	Canola	4	1	
19	RM 319	NW 23 31 20 W3	Canola	5	1	
24	RM 320	NE 36 31 23 W3	Canola	4	1	
22	RM 320	NE 4 33 22 W3	Canola	5	2	
21	RM 320	SW 30 33 22 W3	Canola	4	5	
28	RM 321	SW 26 33 26 W3	Canola	4	3	
32	RM 322	NE 13 32 29 W3	Canola	4	4	
34	RM 322	SE 14 33 29 W3	Canola	5	1	
31	RM 322	SW 17 33 27 W3	Volunteer canola	4	6	
30	RM 352	SE 11 34 27 W3	Canola	4	1	
29	RM 352	SW 5 34 26 W3	Canola	4	2	
7	RM 376	n/a	Canola	2.6 - 4	1	2
10	RM 377	NE 33 39 15 W3	Canola	4	2	
12	RM 377	SE 6 40 15 W3	Canola	2.6 - 4	1	
29	RM 379	NW 29 38 19 W3	Canola	2.8 - 4	2	
22	RM 381	SE 21 38 24 W3	Canola	2.8 - 4	3	
20	RM 381	SE 6 38 25 W3	Mustard	4		1
19	RM 382	SE 24 38 28 W3	Canola	2.6 - 3	2	
13	RM 411	NW 8 41 25 W3	Canola	2.8 - 3	1	
34	RM 434	SW 15 43 7 W3	Canola	5	4	
33	RM 434	SW 16 44 7 W3	Canola	5	2	
35	RM 434	SW 4 44 6 W3	Canola	5	3	
36	RM 434	NE 28 44 5 W3	Canola	5	3	
30	RM 435	SW 16 42 8 W3	Canola	4	3	
25	RM 436	NE 22 44 12 W3	Canola	5	2	
26	RM 436	NW 22 44 12 W3	Canola	5	1	
27	RM 436	SW 5 45 11 W3	Canola	5	1	
24	RM 437	NW 20 44 14 W3	Canola	5	1	
21	RM 437	NW 19 45 16 W3	Canola	5	2	2
18	RM 438	NE 20 44 18 W3	Canola	4	1	
20	RM 438	SW 28 43 17 W3	Canola	5	1	
3	RM 442	SE 35 44 27 W3	Canola	4	2	
5	RM 442	SE 6 46 26 W3	Canola	4		1
16	RM 470	SE 2 47 21 W3	Canola	4	1	
10	RM 471	NE 30 49 24 W3	Canola	4		1
11	RM 471	SW 4 50 23 W3	Canola	4	5	
9	RM 471	SW 9 48 24 W3	Canola	4	1	
1	RM 472	NE 10 46 28 W3	Canola	4		2
7	RM 472	NW 7 49 28 W3	Canola	4	1	
6	RM 472	SW 17 47 26 W3	Canola	4		2

Table 3. Mean mortality (%) and total leaf area consumed (mm^2) by western Canadian *Plutella xylostella* larvae hatched from eggs and fed leaf disks from *Brassica napus* transformed with GFP and Bt (GT *B. napus* lines), and non-transformed *B. napus* cv Westar leaf disks which were untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data presented for 5-7, 10-14, and 18-21 days post-exposure.

Treatment	n	% Mortality			Consumption (mm^2)		
		5-7 d	10-14 d	18-21 d	5-7 d	10-14 d	18-21 d
Westar 'dry'	187	8.02 E	40.22 D	75.42 C	11.37 A	175.85 A	183.66 AB
Westar 'wet'	263	19.01 D	57.72 C	87.19 B	12.60 A	134.94 B	161.64 BC
GFP 2(1)	220	19.55 D	32.23 D	63.31 D	12.07 A	149.70 B	186.62 A
GFP 2(2)	36	2.78 E	58.33 C	97.22 A	8.89 B	135.64 B	147.22 C
GT 1(2)	66	96.97 A	100.00 A	100.00 A	0.10 C	0.12 D	0.12 E
GT 2(1)	54	98.15 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 2(3)	62	87.10 B	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 2(4)	59	100.00 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 2(5)	37	100.00 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 4(7)	59	98.31 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 5(1)	64	100.00 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 5(2)	18	72.22 C	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 6(3)	65	98.46 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 7(2)	54	96.30 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 8(3)	125	100.00 A	100.00 A	100.00 A	0.21 C	0.21 D	0.26 E
GT 8(10)	78	100.00 A	100.00 A	100.00 A	0.10 C	0.12 D	0.12 E
GT 9(1)	56	100.00 A	100.00 A	100.00 A	0.12 C	0.12 D	0.12 E
GT 9(6)	74	100.00 A	100.00 A	100.00 A	0.11 C	0.12 D	0.12 E
GT 9(11)	57	100.00 A	100.00 A	100.00 A	0.11 C	0.11 D	0.11 E
GT 10(2)	60	100.00 A	100.00 A	100.00 A	0.32 C	0.32 D	0.32 E
LD_{50}	185	22.16 D	82.46 B	97.66 A	6.96 B	92.14 C	95.44 D
LD_{95}	177	84.75 B	100.00 A	100.00 A	0.80 C	0.49[1] D	0.49 E

Means followed by the same letter within a column are not significantly different from one another at P= 0.05.

[1] lower consumption value resulted from mortality during the time period

Table 4. Mean mortality (%) and total leaf area consumed (mm^2) by western Canadian *Plutella xylostella* 4th instar larvae fed leaf disks from *Brassica napus* transformed with GFP and Bt (GT *B. napus* lines), and non-transformed *B. napus* cv Westar leaf disks which were untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data presented for 5-7 and 10-14 days post-exposure.

Treatment	n	% Mortality		Consumption (mm^2)	
		5-7 d	10-14 d	5-7 d	10-14 d
Westar 'dry'	132	6.98 I	10.28 F	106.21 A	100.22[1] A
Westar 'wet'	144	17.14 I	19.63 F	96.20 A	95.61[1] AB
GFP 2(1)	104	12.50 I	19.72 F	80.79 B	80.00 C
GFP 2(2)	48	8.70 I	17.14 F	74.66 B	62.38[1] D
GT 1(2)	57	80.70 CDE	91.43 AB	3.32 C	1.16[1] E
GT 2(1)	36	86.11 ABC	97.22 A	0.12 C	0.12 E
GT 2(3)	60	81.67 BCDE	94.44 A	2.55 C	0.12[1] E
GT 2(4)	34	55.88 G	70.59 D	0.11 C	0.11 E
GT 2(5)	36	66.67 FG	88.89 ABC	0.80 C	0.80 E
GT 4(7)	36	83.33 BCDE	100.00 A	0.12 C	0.12 E
GT 5(1)	36	63.89 FG	72.22 D	0.80 C	0.80 E
GT 6(3)	47	65.96 FG	80.00 BCD	0.37 C	0.46 E
GT 7(2)	36	72.22 DEF	97.22 A	0.12 C	0.12 E
GT 8(3)	59	86.44 ABC	91.67 AB	5.06 C	0.46[1] E
GT 8(10)	48	71.74 EF	76.47 CD	0.91 C	1.19 E
GT 9(1)	36	94.44 AB	100.00 A	0.12 C	0.12 E
GT 9(6)	60	76.67 CDEF	91.67 AB	2.95 C	0.80[1] E
GT 9(11)	36	97.22 A	97.22 A	0.12 C	6.93 E
GT 10(2)	45	84.44 ABCDE	100.00 A	2.01 C	0.12[1] E
LD_{50}	131	38.17 H	54.21 E	76.04 B	83.92 BC
LD_{95}	131	84.73 ABCD	88.89 ABC	1.88 C	0.91[1] E

Means followed by the same letter within a column are not significantly different from one another (P= 0.05)

[1] lower consumption value resulted from mortality during the time period

Table 5. Mean mortality (%) and total leaf area consumed (mm^2) by western Canadian *Plutella xylostella* 4th instar larvae fed leaf disks from non-transformed *B. napus* cv Westar leaf disks which were untreated ('dry'), or from GFP-Bt transformed *Brassica rapa* second back-cross with parental line (BC_2) populations from California (CA), and non-transformed *B. rapa* California (CA) population leaf disks which were dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data for observation period 1 (5-7 days) post-exposure. Data presented for 5-7 days post-exposure; leaf material was limited and assay time was shortened.

Treatment	n	% Mortality		Consumption (mm^2)	
		5-7 d	10 - 14 d	5-7 d	10 - 14 d
CA 'dry'	10	0.00 D	n/a	117.75 A	n/a
CA 'wet'	12	0.00 D	n/a	88.93 A	n/a
BC_2a (CA x (CA x (CA x GT 2)))	12	75.00 B	n/a	10.22 B	n/a
BC_2b (CA x (CA x (CA x GT 5)))	11	81.82 AB	n/a	8.92 B	n/a
LD_{50} CA	8	50.00 C	n/a	27.60 B	n/a
LD_{95} CA	12	100.00 A	n/a	11.24 B	n/a

Means followed by the same letter within a column are not significantly different from one another (P= 0.05)

Table 6. Mean mortality (%) and total leaf area consumed (mm^2) by western Canadian *Plutella xylostella* larvae hatched from eggs and fed leaf disks from non-transformed *B. napus* cv Westar which were untreated ('dry'), or from GFP-Bt transformed *B. rapa* first back-cross with parental line (BC_1) and second back-cross with parental line (BC_2), and non-transformed *B. rapa* California (CA) and Quebec (QB2) population leaf disks which were untreated ('dry'), dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data presented for 5-7, 10-14, and 18-21 days post-exposure.

Treatment	n	% Mortality			Consumption (mm^2)		
		5-7 d	10-14 d	18-21 d	5-7 d	10-14 d	18-21 d
CA 'dry'	43	13.95 D	18.61 D	8.70 C	12.69 A	175.14 A	266.64 A
CA 'wet'	38	13.16 D	28.95 D	22.73 B	10.81 AB	128.95 B	253.95 A
BC_1 (CA x (CA x GT 2)	24	100.00 A	100.00 A	100.00 A	0.12 E	0.12 D	0.12 C
BC_2a (CA x (CA x (CA x GT 2)))	23	100.00 A	100.00 A	n/a	0.12 E	0.12 D	n/a
BC_2b (CA x (CA x (CA x GT 5)))	22	100.00 A	100.00 A	n/a	0.12 E	0.12 D	n/a
LD_{50} CA	43	18.61 D	51.16 C	90.48 A	11.13 AB	64.33 C	77.40 B
LD_{95} CA	41	88.10 AB	100.00 A	100.00 A	3.28 CD	3.28 D	3.40 C
QB2 'dry'	18	55.56 C	55.56 C	n/a	12.98 A	60.68 C	n/a
QB2 'wet'	18	83.33 B	83.33 B	n/a	8.89 B	25.25 D	n/a
BC_2a (QB2 x (QB2 x (QB2 x GT 2)))	20	100.00 A	100.00 A	n/a	0.12 E	0.12 D	n/a
BC_2b (QB2 x (QB2 x (QB2 x GT 5)))	17	100.00 A	100.00 A	n/a	0.12 E	0.12 D	n/a
LD_{50} QB2	21	90.48 AB	100.00 A	n/a	5.32 C	5.32 D	n/a
LD_{95} QB2	21	100.00 A	100.00 A	n/a	0.70 DE	0.70 D	n/a

Means followed by the same letter within a column are not significantly different from one another (P= 0.05).

Table 7. Plant weights (vegetative and reproductive components, and reproductive component expressed as proportion of total plant weight) of *Brassica napus* plants transformed with GFP and Bt (GT *B. napus* lines), and non-transformed *B. napus* cv Westar grown under zero or high insect pressure from diamondback moth, *Plutella xylostella* in 2002. Values shown are weight per plant for 10 plants harvested separately (individual) and weight per plant for the total number of plants per cage (cage).

			Saskatoon 2002											
			Individual Plant Weights											
Plant Line	# Plants		VEG				REP				REP as % of TOTAL			
			zero	high			zero	high			zero	high		
	zero	high	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.
Westar	40	40	8.18 A	5.62 A	2.19	*	10.70 A	5.60 B	3.13	**	56.48 B	44.20 B	4.83	**
GFP	40	40	7.90 A	7.68 A	0.13	ns	11.99 A	4.56 B	3.77	**	60.55 AB	32.97 C	8.64	**
GT-6(3)	240	240	7.16 A	8.93 A	-2.24	*	14.12 A	15.46 A	-1.07	ns	66.43 A	64.20 A	1.98	*
			Cage Weights											
Plant Line	# Plants		VEG				REP				REP as % of TOTAL			
			zero	high			zero	high			zero	high		
	zero	high	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.
Westar	160	159	6.19 A	5.33 A	1.10	ns	8.55 C	5.22 B	2.57	ns	57.54 A	49.76 B	7.32	**
GFP	154	152	6.84 A	5.98 A	1.11	ns	10.18 B	3.75 C	3.99	*	59.67 A	33.50 C	2.57	ns
GT-6(3)	947	950	7.23 A	7.29 A	-0.12	ns	12.64 A	12.83 A	-0.27	ns	63.80 A	63.84 A	-0.03	ns

Plant weight data were log-transformed prior to analysis to ensure heteroscedasticity, and then analyzed by the PROC GLM procedure (SAS Institute, 1995).
Differences between means (in columns) were tested for significance with Waller-Duncan t-test. Means labelled with the same letter are not significantly different at P = 0.05.
t values (in rows) derived from Student's paired t-test; * denotes significance at 0.05, ** denotes significance at 0.01.

Table 8. Plant weights (vegetative and reproductive components, and reproductive component expressed as proportion of total plant weight) of *Brassica napus* plants transformed with GFP and Bt (GT *B. napus* lines), and non-transformed *B. napus* cv Westar grown under zero or low insect pressure from diamondback moth, *Plutella xylostella* in 2001. Values shown are weight per plant for 10 plants harvested separately (individual) and weight per plant for the total number of plants per cage (cage).

Saskatoon 2001

Individual Plant Weights

Line	# Plants		VEG				REP				REP as % of TOTAL			
			zero	high			zero	high			zero	high		
	zero	low	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.
Westar	40	39	6.49 B	5.62 A	1.16	ns	10.99 A	9.17 B	1.08	ns	56.88 A	56.67 BC	0.00	ns
GFP	39	40	7.10 B	5.11 A	1.64	ns	13.17 A	10.13 B	1.32	ns	64.01 A	61.40 AB	1.20	ns
GT-1(2)	40	40	8.58 AB	8.03 A	0.42	ns	17.54 A	15.15 AB	0.81	ns	63.45 A	61.33 AB	0.82	ns
GT-4(7)	40	39	6.74 B	6.90 A	-0.06	ns	15.46 A	13.86 AB	0.60	ns	62.85 A	56.41 C	1.43	ns
GT-5(1)	39	40	7.61 AB	7.09 A	0.29	ns	15.46 A	14.42 AB	0.25	ns	61.73 A	63.60 A	-0.74	ns
GT-6(3)	40	38	5.78 B	7.00 A	-1.32	ns	13.79 A	17.77 A	-1.69	ns	63.16 A	69.22 A	2.18	*
GT-7(2)	40	40	11.00 A	8.15 A	1.47	ns	18.93 A	14.78 AB	1.00	ns	57.05 A	53.97 C	0.90	ns
GT-8(10)	40	40	8.36 AB	8.08 A	0.24	ns	16.81 A	15.66 AB	0.34	ns	63.48 A	61.66 AB	0.73	ns

Cage Weights

Line	# Plants		VEG				REP				REP as % of TOTAL			
			zero	low			zero	low			zero	low		
	zero	low	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.	$\bar{x}$	$\bar{x}$	t	sig.
Westar	40	39	4.83 A	4.13 B	0.73	ns	7.33 B	5.77 C	0.97	ns	60.28 BC	57.49 AB	1.3	ns
GFP	39	40	4.98 A	4.26 AB	0.64	ns	9.29 AB	7.50 ABC	3.33	*	65.55 ABC	64.10 AB	0.34	ns
GT-1(2)	40	40	4.49 A	5.41 AB	0.09	ns	10.02 AB	9.24 ABC	0.50	ns	64.31 ABC	63.33 AB	1.07	ns
GT-4(7)	40	39	5.36 A	4.90 AB	0.99	ns	11.01 AB	9.13 ABC	1.06	ns	67.09 AB	61.79 AB	1.04	ns
GT-5(1)	39	40	5.97 A	6.40 A	-0.97	ns	12.11 A	12.36 AB	-0.17	ns	66.65 AB	65.37 AB	0.71	ns
GT-6(3)	40	38	5.16A	5.07 AB	0.10	ns	10.87 AB	13.70 A	-2.26	ns	67.83 A	73.71 A	-2.4	ns
GT-7(2)	40	40	6.23 A	5.45 AB	0.91	ns	9.84 AB	8.35 BC	0.74	ns	59.54 C	56.06 B	0.51	ns
GT-8(10)	40	40	5.52 A	6.01 AB	-0.72	ns	10.56 AB	10.28 ABC	0.11	ns	65.07 ABC	62.28 AB	0.82	ns

Plant weight data were log-transformed prior to analysis to ensure heteroscedasticity, and then analyzed by the PROC GLM procedure (SAS Institute, 1995).

Differences between means (in columns) were tested for significance with Waller-Duncan t-test. Means labelled with the same letter are not significantly different at P = 0.05.

t values (in rows) derived from Student's paired t-test; * denotes significance at 0.05, ** denotes significance at 0.01.

Table 9. Number of seedlings screened and putative F_1 hybrids detected in field experiment (Saskatoon 2001, 2002) with *B. rapa* cultivar AC Parkland acting as the pollen recipient, and transgenic GFP-BT *B. napus* acting as the pollen donor, based on detection of a GFP marker and morphological characteristics (presence of hairs).

Year	No. of Plants	No. of Seedlings	No. of hybrids (GFP)	No. of hybrids (morphology)	% of hybrids	
					Mean ± SE	Range
2001	22	2881	176	176	7.06 ± 2.1	0.0 - 32.7
2002	22	4230	51	51	1.20 ± 0.3	0.0 - 5.6

Table 10. Total leaf area consumed (mm^2) and % mortality of western Canadian *Plutella xylostella* hatched from eggs and fed leaf disks from putative hybrids of *Brassica rapa* × *Brassica napus* (1-2 *etc.*) obtained in field experiment in 2001, *B. napus* containing the *GFP* gene, and non-transformed *B. rapa* cv AC Parkland leaf disks which were untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data for days 7, 14 and 21 post-exposure.

Treatment /line	n	Leaf Area Consumed (mm^2)			% Mortality		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Parkland dry	11	5.07 BC	72.53 B	72.53 B	45.46 B	72.73 B	81.82 B
Parkland wet	12	3.14 CD	65.48 B	75.70 B	50.00 B	66.67 B	83.33 B
GFP	11	17.30 A	278.22 A	318.36 A	9.10 C	18.18 C	54.55 C
1-2	12	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
2-1	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
3-1	10	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
4-1	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
6-1	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
7-2	12	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
8-1	10	0.11 E	0.11 C	0.11 C	100.00 A	100.00 A	100.00 A
9-2	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
11-1	10	0.11 E	0.11 C	0.11 C	100.00 A	100.00 A	100.00 A
12-1	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
13-1	12	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
14-1	9	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
15-3	10	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
17-3	8	0.11 E	0.11 C	0.11 C	100.00 A	100.00 A	100.00 A
19-3	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
21-3	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
22-1	11	0.12 E	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
LD_{50}	10	6.77 B	18.42 C	18.42 C	40.00 B	90.00 A	90.00 AB
LD_{95}	11	0.67 DE	1.22 C	1.22 C	90.91 A	100.00 A	100.00 A

Means followed by the same letter within a column are not significantly different from one another (P=0.05).

Table 11. Total leaf area consumed (mm^2) and % mortality of western Canadian *Plutella xylostella* 4th instar larvae fed leaf disks from putative hybrids of *Brassica rapa* × *Brassica napus* (1-2 *etc.*) obtained in field experiment in 2001, *B. napus* containing the *GFP* gene, and non-transformed *B. rapa* cv AC Parkland leaf disks which were untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data for days 7 and 10 post-exposure.

Treatment /line	n	Leaf Area Consumed (mm^2)		% Mortality	
		Day 7	Day 10	Day 7	Day 10
Parkland dry	10	90.15 C	81.96 C	10.00 D	18.18 C
Parkland wet	11	154.99 B	154.99 B	27.27 D	27.27 C
GFP	11	187.33 A	187.33 A	0.00 D	0.00 D
1-2	12	6.14 E	6.14 E	75.00 ABC	100.00 A
2-1	12	2.62 E	2.62 E	58.33 C	91.67 AB
3-1	12	6.13 E	6.13 E	83.33 ABC	100.00 A
4-1	11	0.66 E	0.60 E	100.00 A	100.00 A
6-1	12	2.09 E	2.09 E	100.00 A	100.00 A
7-2	12	4.13 E	4.13 E	66.67 BC	83.33 AB
8-1	10	2.52 E	2.29 E	70.00 ABC	100.00 A
9-2	12	1.59 E	1.59 E	75.00 ABC	91.67 AB
11-1	12	3.12 E	3.12 E	91.67 AB	100.00 A
12-1	12	2.64 E	2.64 E	66.67 BC	91.67 AB
13-1	12	4.12 E	4.12 E	66.67 BC	83.33 AB
14-1	12	2.12 E	2.12 E	83.33 ABC	100.00 A
15-3	11	4.49 E	4.49 E	81.82 ABC	100.00 A
17-3	12	2.12 E	2.12 E	100.00 A	100.00 A
19-3	11	0.67 E	0.67 E	90.91 AB	100.00 A
21-3	12	1.11 E	1.11 E	83.33 ABC	100.00 A
22-1	12	0.62 E	0.62 E	83.33 ABC	83.33 AB
LD_{50}	12	44.47 D	44.47 D	66.67 BC	75.00 B
LD_{95}	12	5.63 E	5.63 E	100.00 A	100.00 A

Means followed by the same letter within a column are not significantly different from one another (P=0.05).

Table 12. Total leaf area consumed (mm^2) and % mortality of western Canadian *Plutella xylostella* hatched from eggs and fed leaf disks from putative hybrids of *Brassica rapa* × *Brassica napus* (2-1 *etc.*)obtained in field experiment in 2002, and non-transformed *B. rapa* cv AC Parkland leaf disks which were untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data for days 7, 14 and 21 post-exposure.

Treatment/ line	n	Leaf Area Consumed (mm^2)			% Mortality		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Parkland dry	10	15.46 A	277.82 A	277.82 A	10.00 C	30.00 C	40.00 B
Parkland wet	9	15.14 A	308.15 A	321.78 A	22.22 C	22.22 C	22.22 C
2-1	11	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
3-1	10	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
4-3	11	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
5-1	10	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
6-3	12	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
7-2	11	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
9-1	11	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
13-3	12	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
14-1	10	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
15-1	12	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
17-2	11	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
20-1	11	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
21-1	12	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
22-1	10	0.12 C	0.12 C	0.12 C	100.00 A	100.00 A	100.00 A
LD_{50}	11	8.16 B	56.67 B	67.82 B	54.55 B	81.82 B	90.91 A
LD_{95}	10	0.11 C	0.11 C	0.11 C	100.00 A	100.00 A	100.00 A

Means followed by the same letter within a column are not significantly different from one another (P=0.05).

Table 13. Total leaf area consumed (mm^2) and % mortality of western Canadian *Plutella xylostella* 4th instar larvae fed leaf disks from putative hybrids of *Brassica rapa* × *Brassica napus* (2-1 *etc.*) obtained in field experiment in 2002, and non-transformed *B. rapa* cv AC Parkland leaf disks which were untreated ('dry'), or dipped in water ('wet') or in the LD_{50} or LD_{95} rate of Safer's BTK. Data for days 7 and 10 post-exposure.

Treatment/ line	n	Leaf Area Consumed (mm^2)		% Mortality	
		Day 7	Day 10	Day 7	Day 10
Parkland dry	12	185.62 A	185.62 A	16.67 D	25.00 CD
Parkland wet	12	168.86 A	168.86 A	16.67 D	16.67 D
2-1	12	1.64 B	1.64 B	100.00 A	100.00 A
3-1	12	1.55 B	1.55 B	100.00 A	100.00 A
4-3	12	2.86 B	2.86 B	100.00 A	100.00 A
5-1	12	2.46 B	2.46 B	75.00 B	83.33 B
6-3	12	1.64 B	1.64 B	100.00 A	100.00 A
7-2	12	2.76 B	2.76 B	100.00 A	100.00 A
9-1	12	2.05 B	2.05 B	100.00 A	100.00 A
13-3	12	1.94 B	1.94 B	100.00 A	100.00 A
14-1	12	1.64 B	1.64 B	100.00 A	100.00 A
15-1	12	4.50 B	4.50 B	100.00 A	100.00 A
17-2	12	2.86 B	2.86 B	100.00 A	100.00 A
20-1	7	1.23 B	1.23 B	100.00 A	100.00 A
21-1	12	1.74 B	1.74 B	100.00 A	100.00 A
22-1	12	4.17 B	4.17 B	100.00 A	100.00 A
LD_{50}	12	156.39 A	156.39 A	33.33 C	33.33 C
LD_{95}	12	4.50 B	4.50 B	91.67 A	100.00 A

Means followed by the same letter within a column are not significantly different from one another (P=0.05).

Table 14. Pollen viability from putative *B. napus* × *B. rapa* F_1 hybrid plants

Year	No. of Plants	No. of Pollen Grains	% Pollen Viability	
			Mean ± SE	Range
2001	39	44104	42.6 ± 1.90	0.8 - 97.2
2002	26	36407	30.9 ± 1.41	3.2 - 58.8

Figure 1. Saskatoon Research Centre 2001 Plot Plan

insect cage for DBM. Each plot will have two cages, one containing insects and one without insects. TOTAL = 64 cages

pathway

-all cages will be hand-seeded

-a 25m2 plot will be seeded to B. napus and when the plants begin flowering, 25 pots of flowering B. rapa will be placed in the B. napus plot

10 m wide border EXCEL + ACSN14

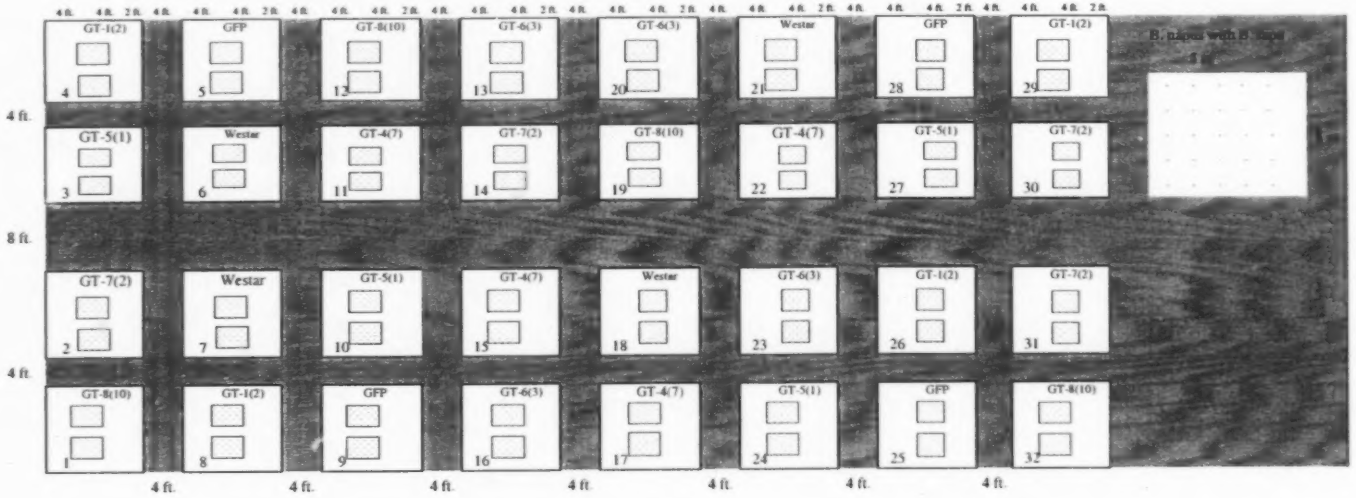


Figure 1. Saskatoon Research Centre 2001 Plot Plan

insect cage for DBM. Each plot will have two cages, one containing insects and one without insects. Total No. = 64 cages

pathway

-all cages will be hand-seeded

-a 25m2 plot will be seeded to B. napus and when the plants begin flowering, 25 pots of flowering B. rapa will be placed in the B. napus plot

10 m wide border EXCEL + ACSN14

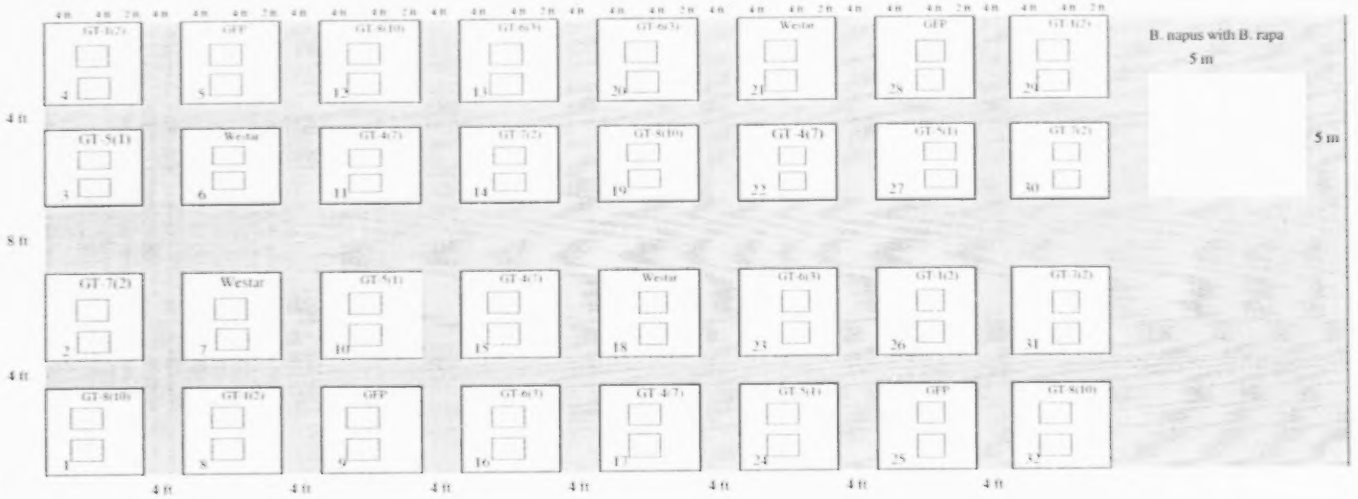


Figure 2. Saskatoon Research Centre 2002 Plot Plan

insect cage for DBM. Each plot will have two cages, one containing insects and one without insects. TOTAL = 64 cages

pathway

-all cages will be hand-seeded

-a 25m2 plot will be seeded to B. napus and when the plants begin flowering, 25 pots of flowering B. rapa will be placed in the B. napus plot

10 m wide border EXCEL + ACSN14

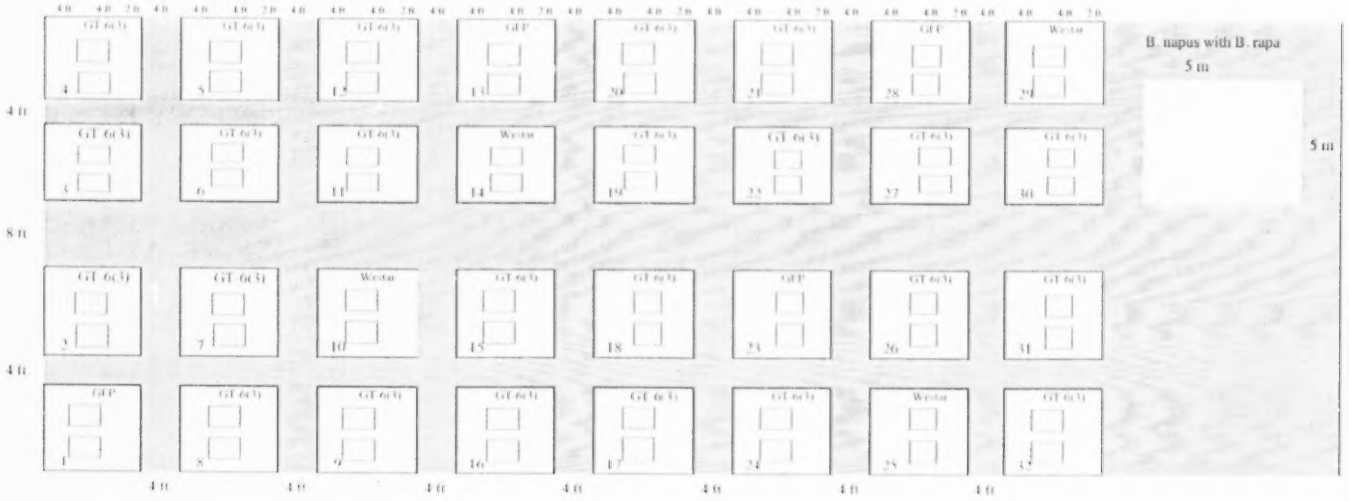


Figure 2. Saskatoon Research Centre 2002 Plot Plan

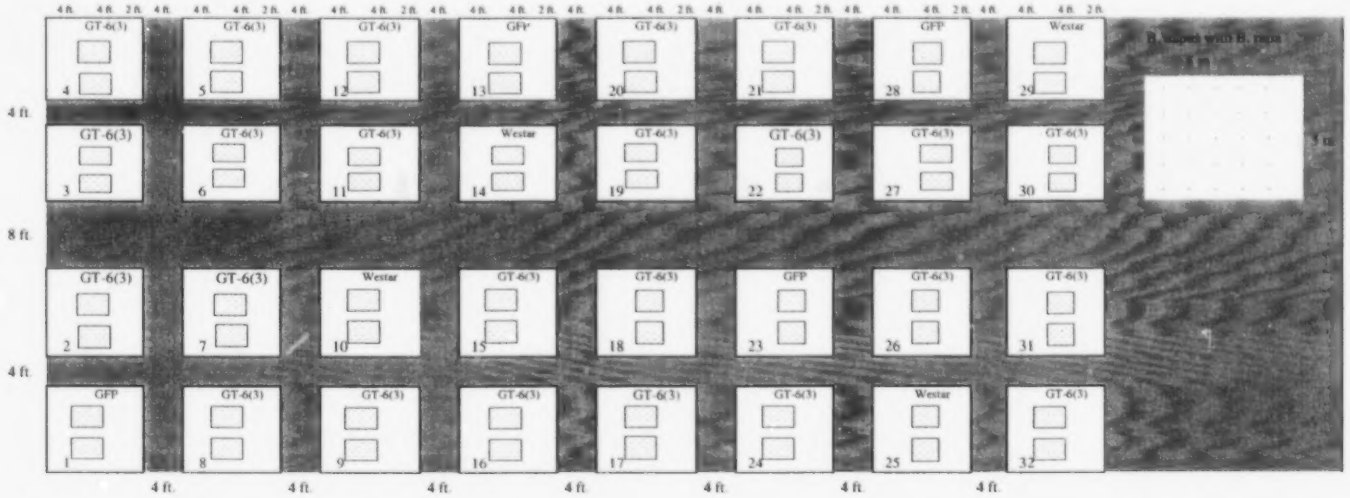
insect cage for DBM. Each plot will have two cages, one containing insects and one without insects. TOTAL = 64 cages

pathway

-all cages will be hand-seeded

-a 25m2 plot will be seeded to B. napus and when the plants begin flowering, 25 pots of flowering B. rapa will be placed in the B. napus plot

10 m wide border EXCEL + ACSN14



Ecological risk of transgenic insect resistance under Canadian field conditions - Field and laboratory assessment of fitness and toxicity of Bt canola

Lorraine Braun[1], Suzanne Warwick[2], Peter Mason[2], Bin Zhu[2,3], and Neal Stewart Jr.[4]

[1]AAFC-SRC, 107 Science Place, Saskatoon SK Canada S7N 0X2; [2]AAFC-CCORC, Ottawa ON Canada K1A 0C6; [3]NWRI-AEPRB, 11 Innovation Blvd., Saskatoon SK Canada S7N 3H5; [4]Dept. Biol. UNC Greensboro NC 27402-6174, USA

Introduction

The Research Branch of Agriculture and Agri-Food Canada is conducting field and laboratory experiments to investigate the movement of insecticidal and *"in vivo* marker" transgenes from canola (*Brassica napus* L. and *Brassica rapa* L.) into two closely related weeds (wild radish, *Raphanus raphanistrum* L. and bird rape, *B. rapa*) via hybridization, introgression and to examine the ecological risk effects of insect resistance to such transgenes under Canadian field conditions. The expression of the insecticidal transgene *cry1Ac* from *Bacillus thuringiensis* (BT) and the marker transgene Green Fluorescence Protein (GFP) from *Aequorea victoria* is being assessed in canola through laboratory feeding bioassays; fitness of transgenic BT-GFP canola is being evaluated in confined field trials; and potential gene flow from transgenic canola into the two closely related weed species is being assessed in separate confined field trials. Research is being conducted in collaboration with the Canadian Food Inspection Agency, Environment Canada, and a USDA funded research team led by Dr. Neal Stewart. We report here on feeding bioassays and fitness experiments conducted in 2000.

Transgenic Canola

(Stewart *et al.*, 1996)

Canola: *Brassica napus* L.

BT gene

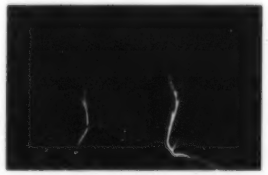
promoter: 34S 5' from cauliflower mosaic virus (CaMV)

gene: synthetic truncated *Bacillusthuringiensis* cry1Ac

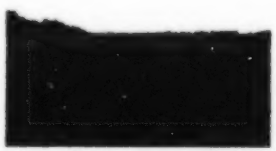
Visible Marker - GFP

promoter: 34S 5' from CaMV

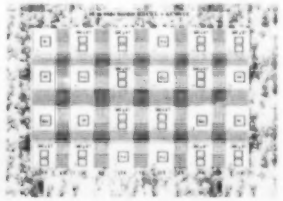
gene: modified green fluorescence protein - endoplasmic reticulum targeted (mGFP5-er) from *Aequorea victoria*



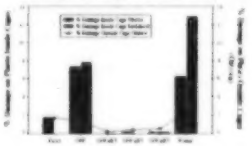
Canola cv. AC Excel | **Canola BTxGFP**

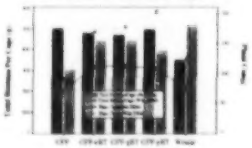


Confined Field Trial Saskatoon 2000



Experimental Design Saskatoon 2000





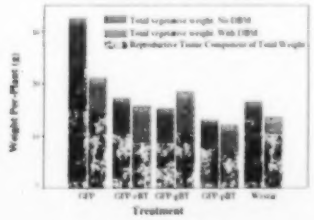
Confined Field Trials

Experimental Design:

The design was a randomized complete block with 4 replicates. The test area was surrounded with a 10 m pollen trap (composed of 2 varieties of *B. napus* canola with different flowering times); pathways were seeded to barley. Treatments included: Excel, Westar, GFP, GT-eBT, GT-gBT, and GT-pBT. The lower case letters in the BT-GFP lines (GT) indicate the degree of fluorescence exhibited by the transformed line: excellent, good, poor. Plots were seeded with 40plants/m^2 and when plants were at the rosette stage, 2 cages were placed in each plot (except for Excel, Westar, and GFP, see plan). DBM of all stages were added to cages in 3 blocks throughout the summer to provide herbivore pressure; one block was chosen at random to contain cages without added DBM.

Feeding damage was rated on leaves from 10 plants per cage twice over the season in 2000 (these plants were harvested for weight). Feeding damage was also rated on plants in plots immediately outside the cages in Ottawa, where there was a natural infestation of DBM.

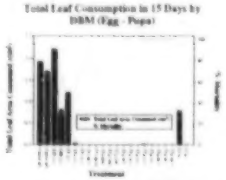
Ten plants were chosen from inside and outside of each cage. Plants were harvested at the end of flowering while still green, and seperated into reproductive (flowering stems, seed pods) and vegetative components. Plant material was dried and weighed to calculate component and total weight/plant. Remaining plants from inside each cage were bulked and seperated into components to calculate total cage biomass.



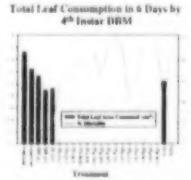
Leaf Disc Bioassays:

Bioassays were conducted to assess feeding damge and mortality on DBM eggs and larvae (4th instar) fed with leaf discs of transgenic BT-GFP canola. Controls included: dry leaf disc, leaf disc moistened with water, LD_{50} dose of Safer's BTK, LD_{95} dose of Safer's BTK.

A minimum of 3 replicates of 24 insects was run for each treatment. Mortality and damage estimates were made on Day 6 (4th instar larvae) and Day 15 (eggs).

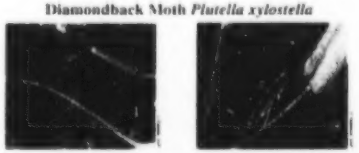


Total Leaf Consumption in 15 Days by DBM (Egg - Pupa)



Total Leaf Consumption in 6 Days by 4th Instar DBM

Diamondback Moth *Plutella xylostella*



Conclusions

- visual scoring of GFP in transformed plants (**e**, **g**, or **p**) was not correlated with BT levels (data not shown)
- feeding damage on BT-GFP canola in cages was negligible
- herbivore pressure from DBM inside cages was insufficient to demonstrate significant effects of BT-GFP on plant biomass per cage
- DBM in cages reduced reproductive tissue weight per plant for GFP and Westar; BT offered protection against yield loss in the BT-GFP lines
- leaf disc bioassays on DBM eggs or 4th instar larvae showed high levels of mortality correlated with low levels of feeding damage as measured by total leaf consumption for all BT-GFP lines except GT-2(7)
- plant line GT-2(7) was not transformed for the BT gene

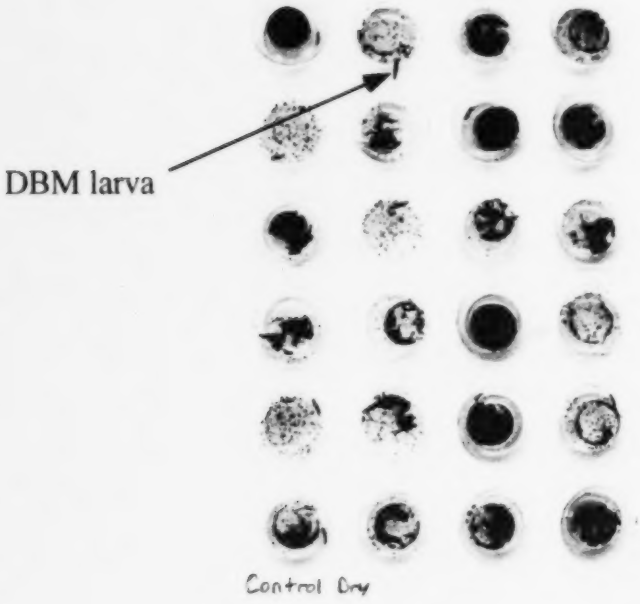
Acknowledgements

This project is funded by the Canadian Biotechnology Strategy Fund and the Saskatchewan Agricultural and Agri-Food Agriculture Development Fund.

We thank Ralph Underwood and Sh-mavin Duff of AAFC for assistance in graphics.

Figure 3. Diamondback Moth Feeding Bioassay

Westar 'dry'



Westar LD_{95}

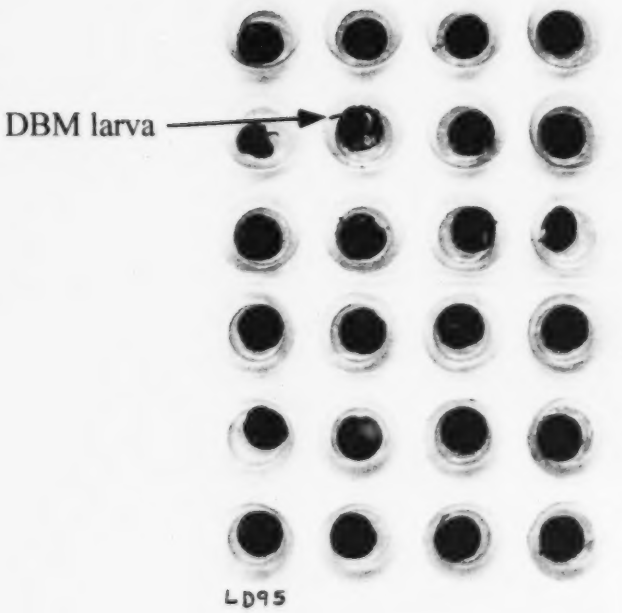


Figure 4. Saskatoon 2001 Field Plot

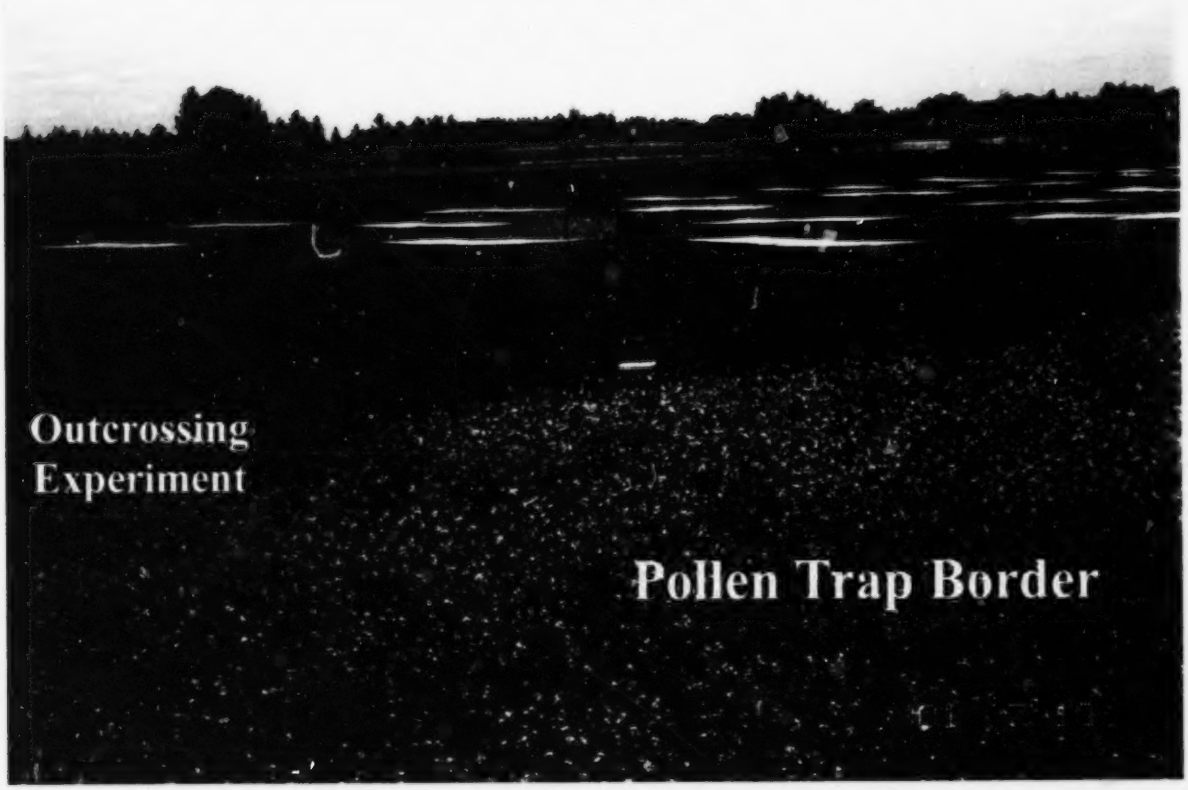


Figure 4. Saskatoon 2001 Field Plot

Figure 5. Saskatoon 2001 Herbivory Experiment

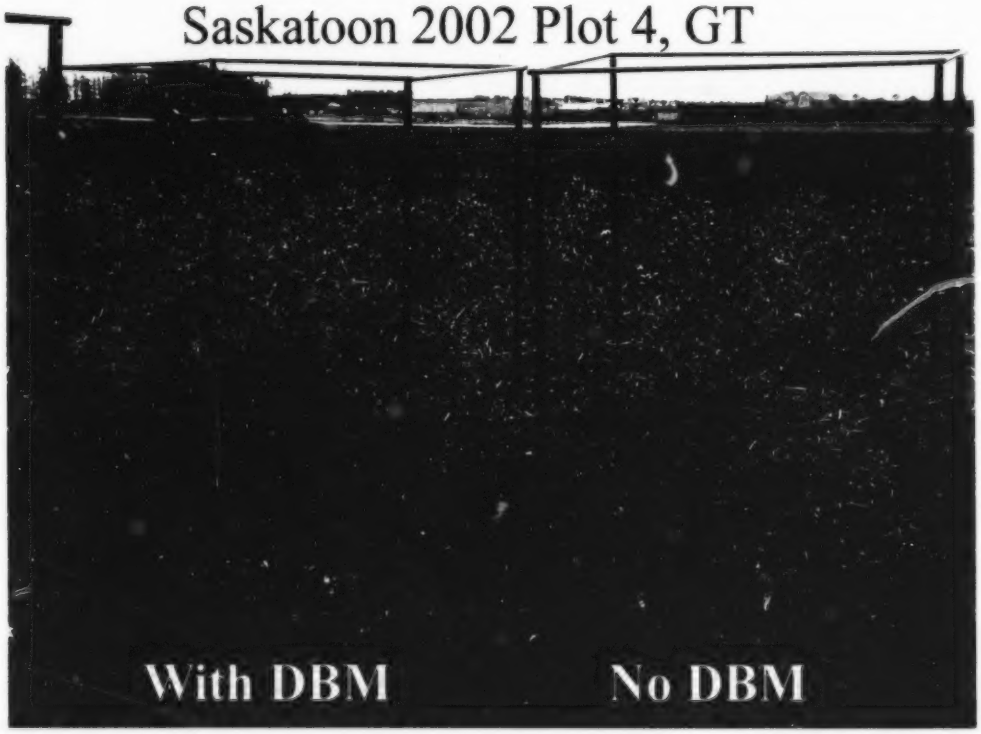
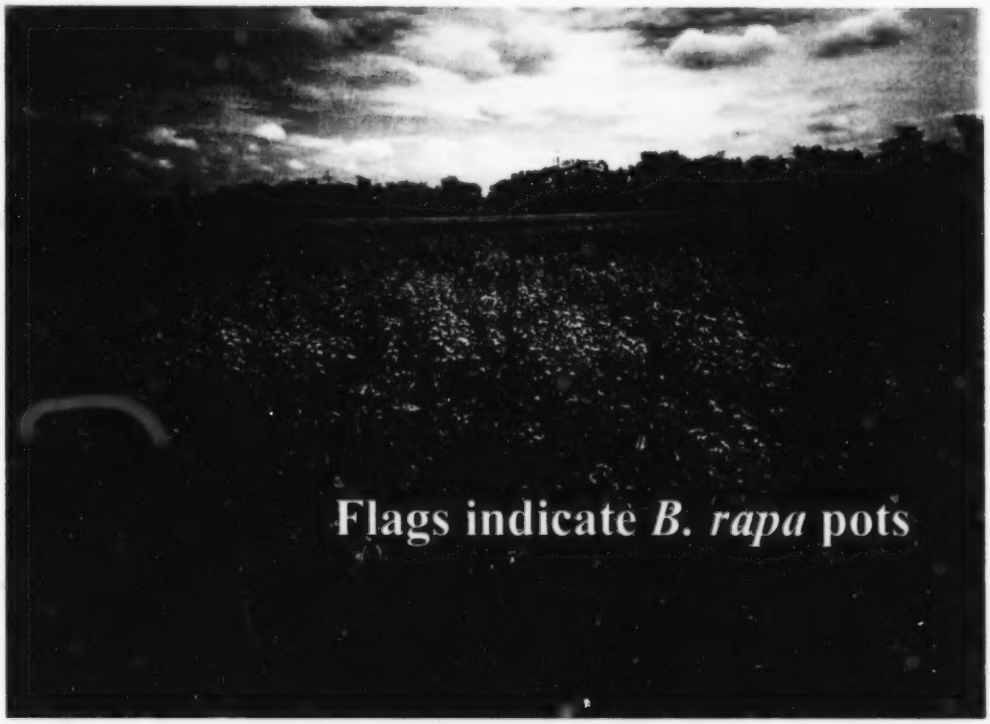
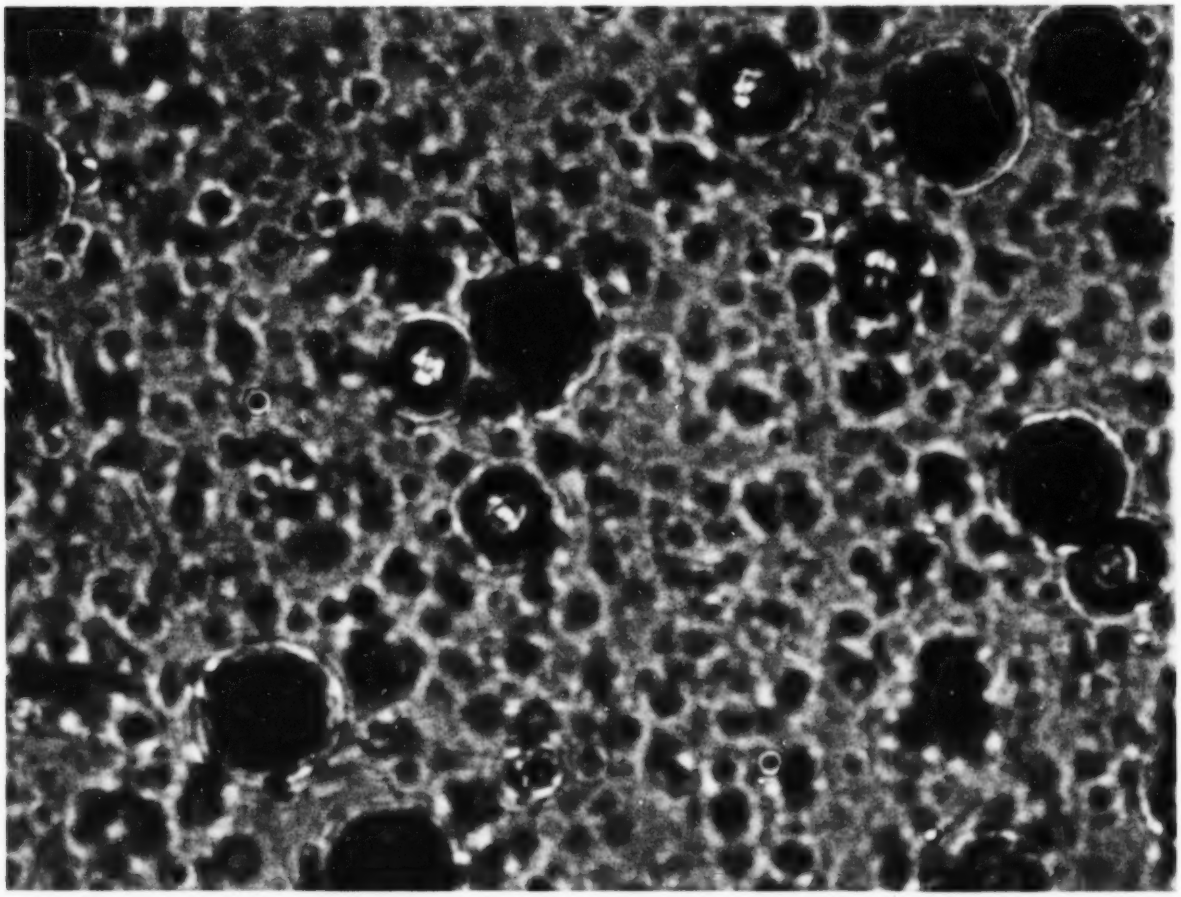


Figure 6. Saskatoon 2001 Outcrossing Experiment



Brassica napus **in ground** ***Brassica rapa*** **in pot**

Figure 7. Pollen Viability from Putative F_1 hybrids



Viable pollen grains stained with 1% aceto-carmine (arrow)